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## HETEROTHALLISM IN SAPROMYCES REINSCHII

### PRELIMINARY NOTE

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(WITH 1 FIGURE)

In 1904, for the first time in the case of the fungi, heterothallism was demonstrated experimentally by Blakeslee (3) in the phycomycetous order Mucorales. Since his epoch making work, this condition of sexuality has been established in the other main groups of fungi by various investigators, their work and the many other important contributions to the complex and significant field of sexuality in the lower plants being assembled and analysed most effectively in the comprehensive survey of Kniep (12).

Although in the zygomycetous series of the Phycomycetes the study of sexual conditions developed rapidly through the work of Blakeslee (4), Burgeff (5) and others, it was not until 1926 that Couch (9) through his significant investigation of heterothallism in *Dictyuchus* of the Saprolegniales extended this study to the Oömycetes.

Yet for research into some of the more complex sexual problems in fungi, the Oömycetes, at least of the aquatic series, are obviously far more advantageous than the Zygomycetes. Their gametangia are more highly and definitely differentiated morphologically into distinctive oogonia and antheridia, while the agents

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of non-sexual reproduction, the zoöspores, in almost all cases, are uninucleate entities permitting isolation and the determining of sex potentialities without the possible complications of the heterocaryotic mingling of nuclei of different sexes which might handicap such studies in the Mucorales. These advantages far outweigh the minor disadvantages that the aquatic Oömycetes are more difficult to isolate, to maintain, and to manipulate, in pure culture, than the terrestrial Zygomycetes.

On surveying the Oömycetes for material especially suited to the investigation of sexuality and related problems *Sapromyces* seemed to the writer particularly promising. This common, although little studied genus of the Leptomitales at present comprises two species, *S. androgynus* Thaxt. and *S. Reinschii* (Schroeter) Fritsch, of which the former, as Thaxter (24) indicated in his original description and as the writer has corroborated in studying material from several points in the United States, from Newfoundland and from Panama, is definitely homothallic (monoecious, androgynous, hermaphroditic—self-fertile). In *S. Reinschii*, however, although the literature (Reinsch 19, Thaxter 23, Petersen 18, Tiesenhausen 25, Minden 15, 16, Graff 10, Apinis 1, Lund 13, Sparrow 22, Cejp 6, 7) still leaves it an open question whether the apparent separation of the sex organs involves a condition actually heterothallic or merely diclinous in the sense of de Bary (2) and other early investigators (*i.e.* male and female organs borne on different parts of the same thallus), preliminary examination in 1921–1926 of material from various sources seemed to the writer to indicate a heterothallic (dioecious, separate-sexed) condition.

If actually heterothallic, *S. Reinschii* would be particularly favorable material for the study of sexuality. Indeed, it would be even more advantageous than the *Dictyuchus* so effectively investigated by Couch. In *Sapromyces* the thallus, of the arbusculate type characteristic of the higher Leptomitales, consists of a cylindrical main axis anchored at its base by sparse, irregular rhizoidal hyphae and giving rise distally to the branches bearing the zoösporangia and sex organs. As a result, the limits of the individual plants can be determined, unhindered by the inextricably intermingled confusion which causes great difficulty and consequent

possibility of error in the Saprolegniales. In addition, not only are the zoospore uninucleate (cf. Kevorkian 11) but on germination the body of the zoospore elongates directly into the main axis so that the origin of individual plants can be traced back directly to the single zoospore source. Moreover, the thallus being constricted at frequent intervals readily becomes plugged off when cut or torn, retaining the content and facilitating dissection of hyphal fragments for the analysis of sex segregation. Finally, there are the minor advantages that the oogonium invariably contains but one egg which is fertilized typically by a single antheridium attaching and sending in its fertilization tube at a definite point, the distal pole of the oogonium.

With these advantages of *Sapromyces Reinschii* in mind a preliminary study of its presumable heterothallism was, at the writer's suggestion, undertaken by Philip H. Jordan in this laboratory from 1927 to 1929. From source material collected from time to time during those years on twigs of *Chamaecyparis thyoides* from a cold spring in a *Chamaecyparis* swamp near Laurelton, New Jersey, Jordan attempted to grow pure cultures on various artificial media but without success. In water cultures, however, on maple twigs, hawthorn fruits and especially on barberries the fungus was easily maintained, growing vigorously, developing zoosporangia and, in dense tufts comprising several plants, in many cases forming oogonia and antheridia as well. As Jordan, despite repeated attempts, was not successful in inoculating individual barberries with single isolated zoospores and inducing these to develop into mature plants, transplanting vigorous basal segments of the main axes of mature plants (cf. FIG. 1,  $A_{10}$ ,  $B_{10}$ ) to separate barberries was next attempted—another method of accomplishing the same end since each basal segment develops directly from the elongation and growth of a single original zoospore. Plants developing oogonia or antheridia were carefully dissected out, the branches and upper segments carefully cut off and each basal segment thus isolated, after thorough washing in sterile water, was carefully transplanted by inserting the base with its few remaining rhizoids in a puncture in a single barberry in a separate sterile water culture. This method proved successful, for with but few exceptions, these transplanted basal segments regenerated vigorous thalli (FIG. 1,  $A_1$ ,  $B_1$ ) which

produced numerous normal zoösporangia. When maintained in separate cultures, however, all these individual plants never formed any oögonia or antheridia. In over twenty such cultures there was but one exception. This, isolation x, derived from the transplanted basal segment of a known female, even when grown by itself did form oögonial initials but did not develop any antheridia, hence its oögonia remained immature and unfertilized.

When, however, barberries bearing the single vigorously growing plants developed from isolated male and female basal segments were placed in the same culture, and the plants were brought together so that the branches were interwoven, sex organs developed in abundance as a result of this contact. On tracing the origin of these it was found that the plants derived from male basal segments had produced antheridia exclusively while those originating from the female basal segments had developed only oögonia. Duplicate plants of the same origin as those used in these tests, but kept separate in water cultures by themselves as controls, grew vigorously and formed abundant zoösporangia but did not develop any sex organs.

The exceptional female strain x mentioned above was at first suspected of being similar to Couch's (9) aberrant female *Dictyuchus* strain N which was parthenogenetic, forming mature oögonia and functional eggs without contact with male plants, yet revealed latent male potentialities by forming antheridia in contact with strongly female strains. Jordan's female strain x, however, gave no evidence for such a suspected apogamy for in single cultures its oögonial initials never matured nor developed eggs. Nor were suspected latent male potentialities revealed, for when grown in contact with various normal female plants, neither participant showed any reaction. Moreover, this exceptional female behaved like the normal female plants when paired with male individuals since it stimulated these to develop numerous antheridial branches, while it produced abundant additional oögonia in which mature oöspores developed as a result of fertilization by the attached antheridia of the male. Except that it formed oögonial initials by itself without needing contact with a male, this exceptional female seemed, therefore, essentially similar to the normal female strains.



Among the many cultures of *Sapromyces Reinschii* grown by Jordan from his Laurelton material collected during 1927-29 there were some which even in the original gross cultures on barberries with dense tufts of several plants intermingled had never formed any sex organs. These corresponded in the characteristics of their thalli and zoösporangia to the nonsexual or "sterile strains" reported by Thaxter (23), Petersen (18), Moore (17), Coker (8), and Matthews (14). It seemed probable, however, from the experiments noted above that they might be plants with sexual potentialities, which by chance had originated from a single zoöspore and hence even in tufts of several individuals were of one sex only. Accordingly, several of these apparently sterile strains were isolated and when their sexual potentiality was tested by pairing them with individuals of known sex, both male and female, in three cases the formation of sexual organs resulted. In one case the supposedly "sterile" strain proved to be female since it showed no response when grown with a plant known to be female, but developed oogonia in contact with a plant known to be male. In the two other cases the "sterile" isolates were found to be male since they did not react in contact with male test plants, but formed antheridial branches and antheridia when grown with known females. These three, one female and two male plants, were in all essential features comparable to the twenty sexual plants previously isolated. There still remained, however, a few of the sterile strains which despite repeated tests never revealed any sexuality. Whether these were indeed sterile, entirely lacking any sex potentiality, or whether they possessed latent very weak sex potentialities which might be evoked by special methods, was not determined.

The foregoing tests by Jordan indicated that *Sapromyces Reinschii* in behavior was predominantly heterothallic in the sense of Blakeslee, the situation, diagrammatically schematized in the accompanying figure (FIG. 1) being as follows: Individual plants, originating from single uninucleate zoöspores are apparently single sexed, either male (*A*) or female (*B*) and when kept separate develop normal vigorous thalli with prolific non-sexual reproduction by zoösporangia and zoöspores ( $A_2-A_n$ ,  $B_2-B_n$ ), but typically (yet note aberrant female strain x) do not form sex organs. On being brought together in pairs (FIG. 1,  $C_1$ ) formation of oogonia

on the female, and of antheridial branches and antheridia on the male, with subsequent fertilization ( $C_2$ ,  $C_3$ ) results if the two participants are of opposite sexes whereas no reaction ensues if they are of the same sex.

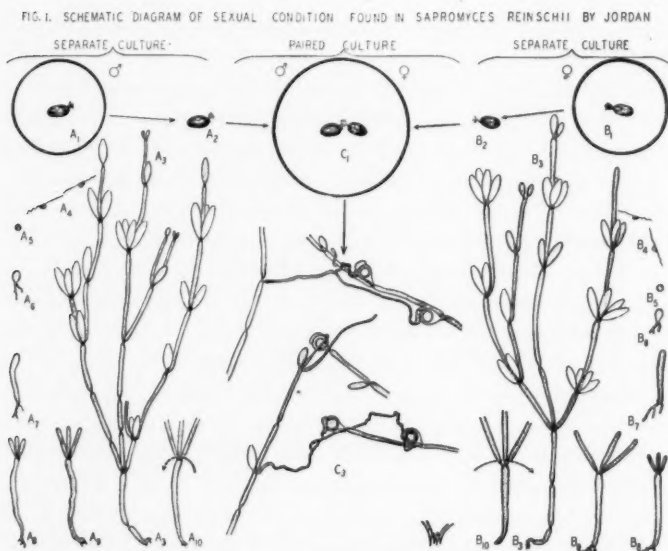


FIG. 1. Schematic diagram of sexual condition found in *Sapromyces Reinschii* by Jordan

This work of Jordan was obviously preliminary, limited by his failure to grow the fungus in pure culture on artificial media, but it should be noted that at that time no one had succeeded in growing *Sapromyces* in such pure cultures even though there seemed no reason *a priori* why this should not be accomplished. The method he used, however, was within its limits thoroughly reliable, since, as has been explained, each individual plant develops directly from a single uninucleate zoospore. The criticism might, of course, be advanced, that tangled in the rhizoidal remnants of the basal segments he isolated and transplanted, there might have been zoospores of another sex which would confuse the results, but it should be noted that the especial care taken to avoid this was apparently successful since duplicate plants kept in separate cultures as controls did not form sex organs.

Even though preliminary, Jordan's work on *Sapromyces Reinschii* brought out several points of interest. It gave experimental evidence of heterothallism in this species, the first demonstration of such a condition in any member of the Leptomitaceae. Moreover, it helped to explain the puzzling "sterile" strains of *Sapromyces* which have been encountered from time to time by showing that some of these may be unisexual plants which by chance have developed under natural conditions without contact with others of the opposite sex. Also, in the case of the exceptional female strain x, it showed that although as a rule the sexual potentiality of an individual remains hidden until evoked by contact with the opposite sex, some females at least, may reveal their sex by forming oögonial initials even when growing alone.

Clearly Jordan's work justified the writer's choice of *Sapromyces Reinschii* as advantageous material with promising potentialities for the investigation of sexuality. From 1933 to the present Harlow Bishop has continued the investigation of this species, securing the fungus for the first time in pure culture on artificial media, successfully isolating and maintaining cultures from single zoöspores, and carrying the problem much farther to obtain results of definite significance revealing a far more complex situation than had been shown previously. Jordan's work has been withheld from publication (save for brief references by Sparrow 20, 21) until repeated and extended, but it now seems timely to present his results in this preliminary note as an introduction to Bishop's detailed and extensive investigation which will soon appear in this journal.

#### SUMMARY

*Sapromyces Reinschii*, an aquatic Phycomycete of the Leptomitaceae, chosen because it possesses distinct advantages for the investigation of sexuality was subjected to a preliminary study by P. H. Jordan in 1927-29. Attempts to grow pure cultures from single zoöspores on artificial media failed, but basal cells, which originate from single zoöspores, when dissected out and transplanted to suitable substrata in water cultures, developed successfully. Predominant heterothallism was revealed, for when such individual plants were kept separate they formed non-sexual organs only, but when male and female individuals were grown to-

gether in contact they produced their respective sex organs and fertilization occurred. A few aberrant cases were encountered, one female, otherwise normal, developing oögonal initials without contact with a male, while several isolates remained apparently sterile showing no reaction to either sex.

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## THE REACTIONS OF THE SWARM-CELLS OF MYXOMYCETES TO NUTRIENT MATERIALS <sup>1</sup>

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In the Myxomycetes it is the swarm-cells which typically initiate the life cycle. The swarmer is organized from the naked protoplast which emerges from the germinating spore, the process in general agreeing with the pioneer description of de Bary (1), although additional features of interest have been contributed by recent studies of germination such as those of Gilbert (5), Smart (9), and others. The swarm-cells are in general more or less tear-drop shaped with a single flagellum at the pointed anterior end, though swarm-cells with more than one flagellum are often observed as pointed out by Gilbert (2). The posterior end is broad and often assumes different shapes even developing short pseudopodia. At the base of the flagellum the nucleus is located and a single round vacuole is usually visible in the posterior portion of the cell.

As the swarm-cells are the significant initial stage in the Myxomycete life cycle their activities are of especial interest. In general they exhibit the following activities: motion, feeding, division, and conjugation. Two types of movement are characteristic of the swarm-cells of most species: one in which the body is jerked into a coma-shape and by the lashing of the flagellum a jerky, rotating movement through the water ensues; and the other in which the swarm-cell ceases its active swimming, settles down, and in an undulatory fashion creeps about over the substratum extending the flagellum its full length and using it as though it were an antenna. These two types of movement may alternate with each other. Division occurs characteristically in well-fed, vigorous swarm-cells by the swarmer retracting its flagellum, rounding off, and dividing into two daughter protoplasts as de-

<sup>1</sup> Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University number 156.

scribed by Gilbert (2). Conjugation may, under favorable conditions, occur while the swarm-cells are in the active rotating stage of motility mentioned above.

As feeding is essential to the life cycle of the Myxomycetes and is of considerable biologic interest in itself because of the simple, presumably primitive, character of the naked swarm-cells, its investigation is of distinct significance.

The swarm-cells of some species such as *Dictydiaethalium plumbeum* may ingest particulate food as shown by Gilbert (3) and others. This ingestion occurs only while the swarm-cells are in the undulatory stage described above. On the other hand, the swarm-cells of such species as *Reticularia Lycoperdon* and *Ceratomyxa fruticulosa* have never been observed to ingest particulate foods. Nevertheless the fact that they increase in size when in culture must mean they derive nutriments in solution.

Yet from a review of the literature it is evident that there is a definite need for more extensive work on the reactions of myxomycetous swarm-cells to available nutrient materials. Accordingly a series of experiments were performed to determine such points as (1) the influence of the nutritive condition of the liquid medium upon the behavior of the swarm-cells especially in relation to the duration of the swarming period and (2) the feeding response of the swarm-cells to particulate food when in nutrient solutions. The results of these experiments are presented in this paper.

#### MATERIALS AND METHODS

The species and varieties of Myxomycetes (shown in table 1) studied in these experiments were the same as those used by the writer (9) in his study of the influence of external factors upon spore germination.

The spores of each species were sown in Syracuse glasses containing 10 cc. of medium. All microscopic observations were made by using a Zeiss \*D water immersion lens. Special methods employed in specific experiments will be described in connection with the discussions of these experiments.



INFLUENCE OF NUTRIENT SOLUTIONS UPON THE  
BEHAVIOR OF THE SWARM-CELLS

That the swarm-cells of Myxomycetes actually make use of food materials is accepted. Different writers, however, disagree as to the manner in which such food is obtained. For example, de Bary in 1887 expressed the opinion that the swarm-cells of Myxomycetes take in food only in solution. In 1890 Lister (6) observed the ingestion and digestion of bacteria by the swarm-cells. Since Lister's observations many authors have noted that the swarm-cells feed upon bacteria and Gilbert (4) showed that, in addition to bacteria, swarm-cells regularly engulf and digest the spores of many fungi encountered in the natural environment of the Myxomycetes, provided the size of these particulate bodies did not exceed one-fifth the volume of the swarm-cell.

Since little definite information is to be found in the literature concerning the comparative influence of nutrients in solution upon swarm-cells of many representative species, and since the writer (9) had found that such nutrient solutions influence spore germination, it seemed of interest to determine whether or not the natural activities of the protoplasts emerging from the spores and the swarm-cells organized therefrom were similarly influenced by the presence of nutrient substances in solution. With this in mind a series of experiments were performed to determine (1) the influence of nutrient solutions upon the time required for the emerged protoplast to become organized into the flagellate swarm-cell, (2) the duration of the swarming period in nutrient solutions, and (3) the end-product of the swarm-cells following the swarming period.

The spores of the species shown in table 1 were sown in the solutions of decoctions of natural substances previously found favorable for the germination of the spores of each species studied (cf. Smart (9) table 1). Preliminary observations of these cultures revealed certain points of interest. For example, the medium which had proved most favorable for the germination of the spores of each species in like manner generally proved most favorable for the activity of the swarm-cells. In some media, however, such as extract of rolled oats, despite the fact that a high percentage of germination was obtained, the protoplasts which had

emerged from the spores were unable to develop further even in very dilute solutions and proceeded immediately to form microcysts. In other solutions such as pea extract, some of the protoplasts slowly metamorphosed into swarm-cells but the swarm period lasted for a time considerably less than in such favorable solutions as extracts of rotten woods. These swarm-cells usually ended in microcyst formation and in no case were they seen to fuse when they had been slowed down in their development. In general, however, the percentage of germination obtained for each species in each medium can be used as a fair index to the reaction of the swarm-cells to the medium. For example, the spores of *Physarum didermoides* gave 30 per cent germination in 8 days in a bean decoction while the same species gave 100 per cent germination in 8 days in decoctions of *Liriodendron* and hay. In the bean decoction the protoplasts required from 30 minutes to 1 hour to put out flagella and swim off in the characteristic fashion and the swarming period continued from 6 to 12 hours ending with the rounding off and encysting of the swarm-cells. While in the decoctions of *Liriodendron* wood and of hay the protoplasts put out flagella in 5 to 20 minutes and the swarming period continued from 24 hours to 7 days; moreover, very few swarm-cells encysted before 3 days or more and the swarming period ceased in many cases following the fusion of the swarm-cells in pairs while in many other swarm-cells the swarming stage ended with the formation of myxamoebae. No myxamoebae were ever observed to put out flagella and become swarm-cells again.

For more intensive study of the influence of nutrient solutions upon the activities of the swarm-cells, the nutrient solution found from these preliminary observations to be most favorable for the normal activity of the swarm-cells of each species was selected and two series of cultures were established. In one of these the spores were sown in the nutrient solution while the second series comprised the controls in which the spores were sown in distilled water. All cultures were maintained at room temperature (18°-22° C.).

The results of these experiments are shown in table 1.

From table 1 it can be seen that the active swarming period of the majority of species is prolonged in nutrient decoctions over

TABLE 1

THE DEVELOPMENT OF SWARM-CELLS IN NUTRIENT MEDIA. NOMENCLATURE IS THAT USED IN MACBRIDE AND MARTIN (7)

Species	Medium	Emergence to swarmer in minutes		Duration of swarming period: hours, days, weeks		End product: zygote, microcyst, myxamoeba	
		In nutrient	In water	In nutrient	In water	In nutrient	In water
<i>Fuligo septica</i> .....	Oak wood	10-15	15-30	1-4w	2d-2½w	z, c, m	c, m
<i>Badhamia utricularis</i> .....	Hay	15-30	20-30	2-4d	2-3d	z, m	c
<i>Badhamia oxyspora</i> .....	Hay	20-35	20-35	2-5d	2-3d	c, m	c
<i>Badhamia rubiginosa</i> .....	Oak leaves	30-40	35-60	4-10h	2-7h	c, m	m
<i>Badhamia liticina</i> .....	Hay	25-40	30-60	2-3d	1-3d	c	c
<i>Badhamia magna</i> .....	Humus	25-40	40-60	1-4d	1-3d	c, m	c
<i>Comatricha nigra</i> .....	Pine wood	12-38	16-52	1-5d	1-2d	z, m, c	c, m
<i>Comatricha elegans</i> .....	Pine wood	15-50	18-82	1-7d	1-4d	z, m, c	c, m
<i>Comatricha typhoides</i> .....	Oak wood	10-38	15-50	1-6d	1-5d	z, m, c	z, m, c
<i>Comatricha pulchella</i> .....	Pine needles	8-68	10-62	1-5d	1-4d	z, m, c	c, m
<i>Lamproderma arcyrionema</i> .....	Pine wood	21-88	30-120	1-4d	1-2d	c, m	c, m
<i>Cribraria intricata</i> .....	Pine wood	10-40	—	7h-2d	—	c	—
<i>Cribraria minutissima</i> .....	Pine wood	11-48	—	6h-1d	—	c	—
<i>Cribraria tenella</i> .....	Pine wood	8-39	15-60	7h-2d	5h-1d	c	c
<i>Cribraria elegans</i> .....	Pine wood	10-50	—	5h-2d	—	c	—
<i>Cribraria aurantiaca</i> .....	Pine wood	8-62	12-91	1-5d	8h-3d	c	c
<i>Dictydium cancellatum</i> .....	Pine wood	15-60	15-60	1-6d	1-4d	c	c
<i>D. cancellatum</i> var. <i>purpureum</i>	Pine wood	14-62	18-61	1-6d	1-4d	c	c
<i>Enteridium Roseanum</i> .....	Pine wood	3-15	5-25	1-14d	1-6d	z, c	c
<i>Reicalaria Lycoperdon</i> .....	Pine wood	3-16	6-35	1-5d	1-14d	z, c	c
<i>Dictydialium plumbeum</i> .....	Pine wood	5-30	5-30	1-4d	1-3d	z, c, m	c, m
<i>Lycogala epidendrum</i> .....	Pine wood	11-90	15-120	1-8d	1-4d	c, m	c, m
<i>Lycogala flavofuscum</i> .....	Liriodendron	8-50	10-60	1-7d	1-3d	c, m	c, m
<i>Perichaena depressa</i> .....	Liriodendron	5-30	8-60	1-11d	1-6d	z, m, c	c, m
<i>Arcyria Oerstedtii</i> .....	Oak wood	12-38	15-60	1-14d	1-7d	z, m, c	c, m
<i>Arcyria nulsans</i> .....	Liriodendron	9-60	10-80	10h-5d	8h-3d	c, m	c, m
<i>Arcyria cinerea</i> .....	Oak wood	10-40	10-40	1-8d	1-5d	z, m, c	z, m, c
<i>Arcyria digitata</i> .....	Oak wood	8-61	20-90	1-7d	1-3d	c, m	c, m
<i>Arcyria densata</i> .....	Pine wood	11-60	12-60	1-14d	1-5d	z, m, c	c, m
<i>Arcyria incarnata</i> .....	Pine wood	10-50	13-61	1-3d	1-2d	c	c
<i>Arcyria pomiformis</i> .....	Pine wood	5-38	10-60	1-5d	1-3d	m, c	c
<i>Oligonema flavidum</i> .....	Pine wood	25-90	30-120	4h-2d	4h-1d	m, c	c
<i>Trichia varia</i> .....	Humus	31-90	30-120	3h-3d	3h-2d	m, c	c
<i>Trichia fatoginea</i> .....	Pine wood	12-70	15-83	6h-5d	4h-3d	z, m, c	m, c
<i>Trichia persimilis</i> .....	Pine wood	15-60	45-120	3h-2d	4h-3d	m, c	c
<i>Physarum cinereum</i> .....	Humus	15-20	25-30	3-8d	1-6d	z, c, m	c
<i>Physarum Serpula</i> .....	Oak leaves	10-20	10-20	2-7d	1-7d	m, c	c
<i>Physarum rubiginosum</i> .....	Oak wood	15-35	20-50	3-10d	1-8d	c, m	c
<i>Physarum globuliferum</i> .....	Oak wood	10-25	20-60	2-6d	1-4d	c, m	c
<i>Physarum pulcherrimum</i> .....	Oak wood	10-30	15-40	4-12d	1-4d	z, c, m	c
<i>Physarum nucleatum</i> .....	Pine wood	30-60	30-60	½-2d	½-2d	c	c
<i>Physarum didermoides</i> .....	Hay	5-20	30-60	1-7d	6-12h	z, c, m	c
<i>Physarum polyccephalum</i> .....	Hay	15-60	30-120	2-7d	1-3d	z, m	z, c, m
<i>Physarum leucophacum</i> .....	Oak wood	30-60	30-60	1-3d	1-2d	m, c	c
<i>Physarum nulsans</i> .....	Humus	10-40	15-60	2-14d	8h-7d	m, c	c
<i>Physarum viride</i> .....	Humus	10-35	15-60	1-5d	5h-3d	z, m, c	c
<i>Physarum flavicomum</i> .....	Willow	10-50	15-180	1-3d	7h-2d	m, c	c
<i>Physarum leucopus</i> .....	Oak wood	15-60	15-120	10h-2d	6h-1d	m, c	c
<i>Physarum digitatum</i> .....	Oak wood	10-90	30-240	3h-1d	2-12h	c	c
<i>Physarum bivalve</i> .....	Humus	30-80	30-180	2-10h	2-10h	c	c
<i>Didymium melanosperrum</i> .....	Pine wood	5-30	15-60	3h-1d	1-8h	z, m, c	m, c
<i>D. nigripes</i> var. <i>xanthopus</i> .....	Humus	20-50	20-50	2-5h	1-3h	z, m	z, m
<i>Didymium squamulosum</i> .....	Hay	5-40	10-50	2-3h	1-3h	z, m	z, m
<i>Craetium leucoccephalum</i> .....	Oak leaves	10-60	20-60	1-7d	1-5d	m, c	m, c
<i>Physarella oblonga</i> .....	Oak wood	5-30	10-40	1-7d	1-4d	z, m, c	m, c
<i>Leocarpus fragilis</i> .....	Oak leaves	10-60	10-60	1-7d	1-5d	m, c	m, c
<i>Diderma testaceum</i> .....	Oak leaves	30-90	30-120	3-6h	3-6h	c	c
<i>Diderma globosum</i> .....	Humus	13-63	30-120	2-8h	2-6h	c	c
<i>Diachea leucopodia</i> .....	Willow	5-40	10-60	1-14d	1-7d	z, m, c	c
<i>Stemonitis fusca</i> .....	Pine wood	5-20	5-30	1-21d	1-10d	z, c	z, c
<i>Stemonitis splendens</i> .....	Hay	10-30	10-45	1-10d	1-7d	z, m, c	c, m
<i>S. splendens</i> var. <i>flaccida</i> .....	Hay	9-29	10-60	1-8d	1-5d	z, m, c	z, c
<i>Stemonitis axifera</i> .....	Willow	5-45	5-60	1-18d	1-4d	z, m, c	c, m
<i>Comatricha laxa</i> .....	Pine wood	8-42	9-42	1-5d	1-3d	c	c
<i>Trichia contorta</i> .....	Pine wood	22-70	30-90	3h-1d	3h-1d	c	c
<i>Trichia floriformis</i> .....	Pine wood	18-62	21-65	12h-3d	8h-1d	m, c	c
<i>Hemitrichia Serpula</i> .....	Pine wood	30-90	30-120	4h-2d	8h-1d	c	c, m
<i>Hemitrichia vesparium</i> .....	Pine wood	15-90	30-120	1-8d	8h-4d	m, c	c, m
<i>Hemitrichia clavaria</i> .....	Pine wood	10-60	21-62	1-5d	1-3d	z, m, c	c, m

that observed in those cultures in which water is used as the medium. Further, the time required for the protoplast to put out a flagellum and assume the typical swarm-cell stage is in general shorter than that in water. The most interesting fact, however, to be derived from consulting the table is that nutrient decoctions favor the fusion of the swarm-cells and stimulate plasmodium formation.

Since the swarm-cells which did not fuse sooner or later became myxamoebae or microcysts, it was thought that the cessation of the swarming period may be due to the accumulation of waste products or the exhaustion or decomposition of usable products in the medium. Therefore, an attempt was made to prolong the active swarming period of the swarm-cells by frequent changes of the nutrient decoction. To test this point *Fuligo septica*, *Enteridium Rozeanum* and *Reticularia Lycoperdon* were chosen because their high percentage of germination insured an immediate supply of material for investigation. Three lots of cultures were established consisting of fifteen cultures each. Lot 1 consisted of spores sown in water and used as controls; in lot 2 the spores were sown in nutrient decoctions; and in lot 3 the spores were sown in nutrient decoctions which were subsequently changed twice daily for a period of two weeks, after which the changes were made every other day. To change the medium each culture was poured into a centrifuge tube and centrifuged for five minutes after which the liquid was decanted off and the remaining swarm-cells were placed in fresh, well aerated nutrient medium. The cultures of lot 1 and of lot 2 were centrifuged each time those of lot 3 were centrifuged and for the same period of time, but the medium was not changed. To insure favorable aeration all cultures were vigorously shaken following centrifuging. The results of these experiments are shown in table 2.

That an adequate supply of fresh medium prolongs the swarming period of the three species shown in table 2 can not be mistaken. Not only is the swarming period prolonged, but division of the swarm-cells is more frequent in the fresh medium than in either water or old medium. Furthermore, fusions of swarm-cells are more abundant in the fresh medium. The centrifuging and subsequent shaking of the cultures seem to have no deleterious

TABLE 2  
EFFECT ON THE DURATION OF THE SWARMING PERIOD ON REPLACING  
OLD NUTRIENT SOLUTIONS WITH FRESH

Species	Duration of swarming period in days					
	Lot 1 (water)		Lot 2 (nutrient)		Lot 3 (changed nutrient)	
	Range	Mode	Range	Mode	Range	Mode
<i>Fuligo septica</i> .....	7-14	12	7-28	25	7-90	80
<i>Enteridium Rozeanum</i> ....	1-6	5	2-15	12	3-28	24
<i>Reticularia Lycoperdon</i> ...	1-5	3	2-14	11	2-21	17

effects upon the swarm-cells other than that of causing them to become slightly more sluggish and to assume more or less abnormal shapes for a short period of time before resuming their normal activity.

In one culture of *Fuligo septica* the medium containing the swarm-cells was left in the centrifuge for two hours after centrifuging with the result that upon returning the culture to its Syracuse glass and examining it under a water immersion lens many pairs of swarm-cells were found partially fused. The process of fusion continued to completion and two days later a beautiful plasmodium had formed which measured  $\frac{1}{2}$  inch or more in its spread condition on the bottom of the dish. This occurrence brought to the writer's mind the possibility of using the centrifuge in promoting the fusion of swarm-cells. The results of a series of experiments performed to test this point will be described in a subsequent paper.

#### THE FEEDING RESPONSE OF SWARM-CELLS TO PARTICULATE FOODS WHEN IN NUTRIENT SOLUTIONS

The ingestion of particulate food by the swarm-cells of Myxomycetes has been observed by several writers since Lister (6) in 1889 first described the ingestion of bacteria. Gilbert (4) extended our knowledge of the feeding habits of the swarm-cells by not only showing that fungous spores and other particulate food as well as bacteria were ingested, but by also describing in detail the method by which the actual ingestion takes place, as follows: when the swarm-cells pass to the undulatory, creeping stage and

come in contact with fungous spores and bacteria, "a tenuous pseudopodium, put out from the posterior part of the body, attaches itself to a spore or bacterium . . . and then retracts, drawing the spore or bacterium towards the body where extensions of protoplasm fold over and enclose it." Miller (8) grew some species of Myxomycetes in hay decoction containing milk and found that the swarm-cells and myxamoebae fed upon the bacteria present in spite of the liquid nutrient available in the medium. In view of the fact that the writer had observed the ingestion of bacteria and spores by the swarm-cells of many species of Myxomycetes not only in water but also in nutrient solutions it seemed desirable to determine if possible whether or not any differences existed between the preferential selection of the particulate food material when the swarm-cells were in nutrient solutions and when in a medium devoid of soluble nutrients. To this end a series of experiments were performed.

As the subjects of these experiments *Dictydiaethalium plumbeum* and *Stemonitis splendens* var. *flaccida* were selected because they had been found to pass quickly to the undulatory stage during which they readily ingested particulate food.

As the solid nutrient to be tested the spores of two Hymenomycetes, *Daedalea quercina* (L.) Fries and *Hydnum septentrionale* Fries and the following bacteria,<sup>2</sup> *B. coli*, *B. subtilis* and *B. prodigiosus*, were chosen. The spores of the Myxomycetes were sown in distilled water in one series and in another in the nutrient decoctions already found most favorable for the Myxomycetes in question. Upon the germination of the spores of the Myxomycetes, the spores of the fungi and the bacteria were added to the cultures.

In the water cultures the swarm-cells of both species of Myxomycetes passed quickly to the undulatory stage and the bacteria and spores were voraciously attacked, the bodies of the swarm-cells becoming gorged therewith. In the cultures of nutrient decoctions the swarm-cells remained in the rotating condition for a much longer period of time but occasionally passed to the creeping stage and ingested both spores and bacteria. In spite of the fact that the bacteria multiplied more freely in the nutrient solution

<sup>2</sup> For authentic determinations of the bacteria employed the writer is indebted to Dr. Aubry Straus of Richmond, Virginia, and to Dr. Dulaney of the Pathological Institute of Memphis, Tennessee.

and the swarm-cells came in contact with them more frequently, it was evident that they were ingested in less numbers than in the water cultures. In nutrient solutions the undulatory stage was brief and occurred at irregular intervals while in water the swarm-cells remained in the creeping stage for hours at a time unless intense illumination was used whereupon they either became quiescent or left the creeping stage and swam off in characteristic rotating fashion. Furthermore, in the nutrient solutions the swarm-cells remained active on the whole for a longer period of time than those which had been feeding upon bacteria and fungous spores in water cultures.

In view of the fact that the period of activity of the swarm-cells was longer in nutrient solutions in which bacteria and fungous spores were present as additional food material than in water cultures in which this particulate food was the only source of nutriment, the behavior of the swarm-cells in media completely free from other organisms seemed worthy of investigation. Therefore spores of *Dictydiaethalium plumbeum* and of *Stemonitis splendens* var. *flaccida* were washed in 1:20,000 mercuric chloride solution, rinsed in sterile water, washed in 1:20,000 hexylresorcinol solution and rinsed through two changes of sterile distilled water and sown in Ehrlenmeyer flasks containing nutrient decoction already found most favorable for activity. The flask cultures were examined by removing drops of the medium containing the swarm-cells by means of sterile pipettes under aseptic conditions in a sterile transfer chamber.

In both species studied, the swarming period was found to be of greater duration in nutrient solutions lacking particulate foods than in any culture containing such food. This seems to indicate that even though bacteria and fungous spores are utilized as food by myxomycetous swarm-cells, they are not necessary for the sustenance of the swarm-cells so long as favorable liquid nutrient materials are present. The presence of bacteria in great numbers is, in fact, detrimental to the normal activity of the swarm-cells since these organisms cause the more rapid accumulation of products unfavorable for the activity of the swarm-cells.

The results of these experiments indicate that the swarm-cells of Myxomycetes make use of nutriment both in the solid and in the



liquid condition. If favorable dissolved nutrients are present in the medium together with bacteria and fungous spores of sorts favorable for ingestion, the swarm-cells derive the bulk of their nutriment from the solution, though they do ingest bacteria and fungous spores to some extent.

#### SUMMARY

Sixty-nine species and varieties of endosporous Myxomycetes representing all types of fruiting bodies known to this group of organisms were studied in an effort to determine the influence of nutrient materials upon the activity of the flagellate swarm-cells. This paper is a report on (1) the influence of the nutritive condition of the liquid medium upon the behavior of the swarm-cells, especially in relation to the duration of the swarming period and (2) the feeding response of the swarm-cells to particulate food when in nutrient solutions.

When grown in weak decoctions of natural substrata such as rotting wood and bark, decaying leaves, hay, humus from mixed woods, etc., the time required for the protoplast which escapes from the spore membrane to put out a flagellum and assume the typical swarm-cell stage is in general shorter than when the swarm-cells are grown in pure water. Further, the active swarming period of the majority of species is prolonged in nutrient decoctions. Of greater interest, however, is the fact that nutrient decoctions favor the fusion of the swarm-cells and stimulate plasmodium formation.

When the swarm-cells of such species as *Dictydiaethalium plumbeum* and *Stemonitis splendens* var. *flaccida* are grown in nutrient decoctions where particulate foods such as bacteria and fungous spores are also available, fewer bacteria and spores are ingested than when only particulate foods are available. Furthermore, the swarming period is of greater duration in nutrient solutions lacking particulate foods than in culture containing such food. The swarm-cells, therefore, make use of nutriment both in the solid and liquid condition, but if favorable dissolved nutrients are available, the swarm-cells derive the bulk of their nutriment from the solution.

## ACKNOWLEDGMENTS

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CHANGES OBSERVED IN CULTURES OF  
ASPERGILLUS NIGER BOMBARDED  
AS SPORES WITH LOW VOLT-  
AGE CATHODE RAYS

R. M. WHELDEN<sup>1</sup>

(WITH 1 FIGURE)

For the past eighteen months the writer has been one of a group who are studying the effects of low voltage cathode rays on living cells. The greater part of the work has been devoted to the ubiquitous black mold, *Aspergillus niger*. This organism was used, first, because it was found that this species would yield cultures in which the spores were extremely uniform in size, of spherical shape, and with a smooth cell wall. Furthermore, each spore had a single nucleus which was almost invariably located centrally. Finally, the spores of this species would tolerate the conditions necessary for radiation, particularly the high vacuum in which radiation was carried on.

The cultures were grown throughout on a potato maltose agar, and were subcultured every two weeks. For radiating, the spores were spread with a dry brush on small highly polished metal slides, so that clumping of spores might be reduced to a minimum. These slides were then placed in a radiation chamber. The air in the chamber was exhausted; and the spores in the middle part of the metal slides radiated, those on the ends serving more or less as controls. As a further control, another slide bearing spores was placed in the center of the vacuum chamber, and shielded from any possible radiation, but subjected to all the other conditions surrounding the radiated spores.

After radiation, the spores were transferred from the slides to the culture medium in Petri dishes. This was done by pressing the spore-bearing side of a slide gently but firmly against the surface

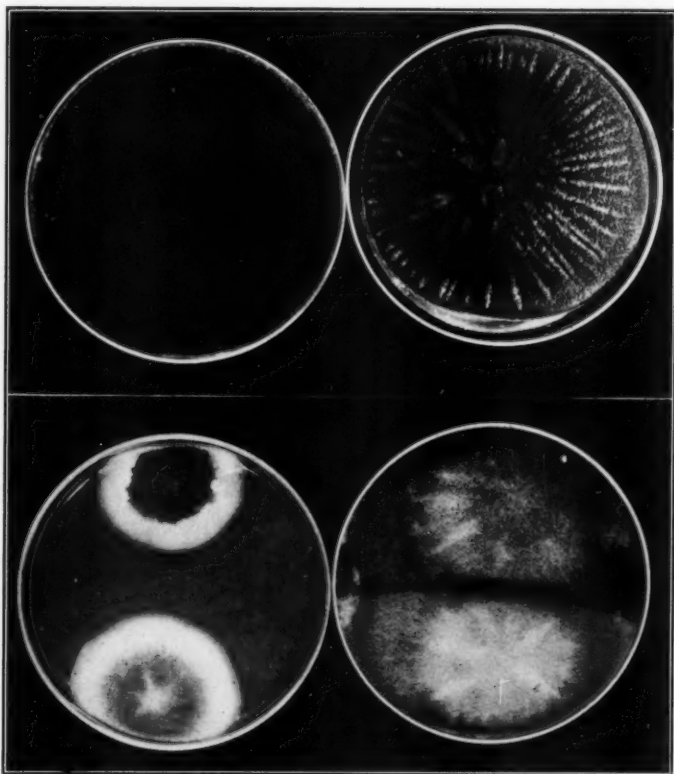
<sup>1</sup> From the Haskins Laboratories, Schenectady, N. Y., and from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, Contribution No. 157.

of the agar and then carefully lifting the slide and leaving the spores on the agar. The spores were then allowed to grow at room temperature for approximately nine hours, after which period statistical observations were made on the number of spores affected by radiation, as indicated by the percentage of spores not germinated. These results will be published later, along with an extensive description of the apparatus. After the statistical counts were completed, spores from the radiated area, from the non-radiated ends of the radiated slides, and from the control slide were transferred from the growing cultures, by means of a fine sterile needle, to separate Petri dishes of agar. These transfers were then set aside until they had grown to maturity, at which time they were examined for possible macroscopic variations which might be due to radiation.

One of the most noticeable results was a pronounced series of radiating ridges which became more numerous as the culture grew out horizontally (FIG.). This phenomenon was undoubtedly due to radiation, as controls, whether from the protected control slide, or from the non-radiated ends, showed at best only the faintest indication of wrinkling, while the radiated spores developed such cultures almost invariably. No wrinkling was found in cultures from non-radiated spores which had been subjected to the high vacuum involved, of the order of  $10^{-5}$  millimeters of mercury, under varying temperatures from  $0^{\circ}$  to  $20^{\circ}$  C., even when grown in different culture-media, or under varying conditions of light. In this wrinkling, the mycelial mat seemed to grow faster tangentially than radially. For this no satisfactory explanation can be offered at present. Examination failed to reveal any apparent change in the microscopic structure of the fungus. The wrinkling did not appear in cultures from spores which had been taken from the wrinkled cultures, indicating that it is not a permanent effect.

The only other result noticed which may have been due to radiation was the occurrence of a mutant, constant through many successive generations, which was strikingly unlike the parent organism (FIG.). Only one such mutant occurred in all the more than three hundred cultures made. It appeared in a culture grown from spores which had received a total dosage of  $31 \times 10^{-9}$  coulombs per square centimeter, with a voltage of 6 kilovolts, having

been exposed for 23 hours to a vacuum of the above-mentioned order. Its most noticeable difference was in color. When the spores first began to appear they gave to the culture a buffy brown



Top, cultures grown from non-radiated spores (left) and radiated spores (right).

Bottom, mutant cultures (above) and normal cultures (below) of *Aspergillus niger* van Tieghem.

tone which gradually darkened with increasing age to Saccardo's umber, and finally, in old cultures, became a deep Prout's brown (colors based on Ridgway's *Color Standards and Nomenclature*). A second, less obvious difference was seen in the greater thickness

and density of the mycelial mat, and in the slower and less abundant development of sporophores. A third distinguishing characteristic was the pronounced earthy odor of the culture, very like that of newly dampened sod. There was no change in the appearance of the conidiophore, or in the dimensions of the spores. This mutant is very like that described by Miss Schiemann under the name of *Aspergillus cinnamomeus* (Zeitsch. f. Induktive Abstamm. und Vererbungslehre, Bd. 8, Heft 1/2, pp. 1-35, 16 fig., 2 pl., 1912).

In the present investigation no other phenomena have appeared which might be attributable to radiation effects. However, work on the problem is only begun, and since this is the first opportunity which has been available to test the effects of low voltage electron beams impinging directly on living material, it is hoped that in the course of further work other effects may be observed.

This work has been done with a low voltage cathode ray tube, the construction of which has been partially financed by a grant generously made by the National Research Council to Prof. Robley D. Evans of Massachusetts Institute of Technology and partially by funds from the Haskins Laboratory.

## MYCOLOGICAL NOTES FOR 1934-35<sup>1</sup>

L. O. OVERHOLTS

(WITH 1 FIGURE)

### FUNGI IMPERFECTI

#### 1. CERCOSEPTORIA LEPTOSPERMA (Peck) Petrak.

On living leaves of *Aralia* sp. Allegheny National Forest, Pa., June 29, 1933. Spores  $40-80 \times 1-1.5 \mu$ , on indistinct pale yellowish green spots 2-7 mm. long and broad. Conidiophores barely protruding through the stomata. Peck describes the spores as slightly enlarged at one end and septate in that region, but in my collection the septa seem to be about equally spaced, usually 3 in number. It is an inconspicuous fungus with somewhat the appearance of a *Microstroma*. The genus *Cercoseptoria* seems almost superfluous and is not to be sharply delimited from either *Ramularia* or *Cercospora*. The practice of separating borderline or intermediate species into new genera has little to commend it.

#### 2. CERCOSPORA OMPHALODES Ellis & Holway.

On living leaves of *Polemonium reptans*. This is an old collection made by J. B. Demaree at State College, Pa., in 1912. The conidiophores are shorter than originally described, being only  $20-30 \mu$  long. Conidia subhyaline to somewhat smoky, finally up to 4-celled,  $32-60 \times 2.5-5 \mu$ .

#### 3. CERCOSPORA SEPTORIOIDES Ellis & Ev.

On living leaves of *Rubus triflorus*, at Seventh Lake, Adirondack Mountains, N. Y., Aug., 1934.

Spots indefinite and irregular, more distinct from below, appearing as yellow flecks above, at first 2-3 mm. broad, then larger

<sup>1</sup> Contribution No. 704, Department of Botany, The Pennsylvania State College, State College, Penna.

For the last previous article in continuation of this series see MYCOLOGIA, 26: 502-515, 1934.



and more indefinite; conidiophores hypophyllous, appearing as a minute straw-colored scurfiness, short,  $20\ \mu$  long,  $2\text{--}3\ \mu$  diameter; conidia elongate,  $60\text{--}80 \times 2.5\text{--}3.5\ \mu$ , often curved and flagellated toward the apex, showing multiseptate condition only after some time in phloxine.

The species was originally described from West Virginia.

4. *CERCOSPORA SQUALIDULA* Peck.

On living leaves of *Clematis virginiana*, Lander and Devil's Tower, Wyo., July 26, 1926.

The species was originally described from New York; reported from Iowa by Holway but not mentioned by Gilman & Archer; and reported from Wisconsin by Davis. Spores  $40\text{--}110 \times 4\text{--}5\ \mu$ .

5. *CERCOSPORELLA FRASERAE* (Ellis & Ev.) Sacc.

On living leaves of *Frasera speciosa*. Yellowstone Park, Wyo., July 21, 1926. Originally described from Colorado. Spores  $80\text{--}120 \times 3.5\text{--}4.5\ \mu$ .

6. *Colletotrichum Trillii* (Sacc.) comb. nov.

On living leaves of *Trillium* sp. Duhring, Forest Co., Pa., June 30, 1933. This was originally described under the untenable name *Vermicularia concentrica* Peck & Clinton. My material, agreeing in other points, seems referable to *Colletotrichum* rather than to *Vermicularia*. Acervuli visible from both surfaces, with some tendency to a concentric arrangement,  $120\text{--}200\ \mu$  diameter, producing spots with a central pallid area surrounded by a pale brown and rather broad margin, and a broad yellowish and indefinite border. Conidia  $16\text{--}24 \times 2\text{--}2.5\ \mu$ ; setae  $4\text{--}6\ \mu$  diameter,  $40\text{--}200\ \mu$  long.

7. *CYLINDROSPORIUM HERACLEI* Ellis & Ev.

On living leaves of *Heracleum lanatum*. Yellowstone Park, Wyo., July 21, 1926. Originally described from Utah. Spores  $40\text{--}60 \times 3\text{--}4\ \mu$ .

8. *CYLINDROSPORIUM KERRIAE* V. B. Stewart.

This species, on *Kerria japonica*, was received from Pittsburgh, Pa., in Sept., 1934. Only the conidial stage was present. It has

not been reported much in the literature. Clinton includes it in his Handbook and Gilman and Archer record it, so that it is probably a species of wide distribution.

9. *ISARIOPSIS ALBOROSELLA* (Desm.) Sacc.

A very small amount of this apparently rare fungus was taken on dead spots in leaves of *Cerastium vulgatum*, at Slippery Rock, Pa., in 1933. The coremia form a white cottony mass on the lower leaf surfaces. Conidiophores faciculated to form a rather loose column, the individuals  $250-280 \times 2.5-3.5 \mu$ , septate, slightly enlarged and geniculate at the apex. Spores elongate, hyaline, 2-celled,  $24-32 \times 5-7 \mu$ .

10. *KELLERMANNIA YUCCAGENA* Ellis & Ev.

On dead flowering stalks of *Yucca*. Chamberlain, S. Dak., July 10, 1926. Spores subcylindric, hyaline or slightly yellowish, 2-celled,  $36-50 \times 11-12 \mu$ , with an apical hyaline straight or laterally bent appendage  $25-30 \mu$  long. These appendages were originally described as basal.

11. *LEMONNIERA AQUATICA* DeWild.

On decaying leaves of *Acer rubrum* in back water of sluggish stream. Ingleby, Center Co., Pa., Nov. 24, 1935. H. A. Wahl. I have found no published record of the occurrence of this species in America. Dr. Linder agrees in assigning the material to this species.

12. *MACROPHOMA RAUI* (Peck) Berlese & Fogl.

On living leaves of *Artemisia tridentata*, at Jackson Lake, Wyo., July 25, 1926. Spores variable and irregular, elongate-ovoid for the most part, hyaline, 1-celled,  $16-24 \times 6-8 \mu$ . The type locality for this species is rather indefinite. The fungus was said to occur on *Artemisia scopulorum*, which is a high altitude plant of the Rocky Mountains. Dearness and House transferred this to *Phyllosticta*; Saccardo to *Phoma*; it was originally described as *Sphaeropsis*.

## 13. OVULARIA ISARIOIDES Sacc.

A peculiar species inhabiting dead irregular spots 1 cm. or more broad, with a dark purple border. Conidiophores strongly fasciculate, forming white cirrhi especially along the veins in the dead area, mostly hypophyllous. Spores  $8-12 \times 3-4 \mu$ , hyaline, 1-celled, produced in chains. Conidiophores roughened at their apexes by the points of attachment of the spores, up to  $160 \mu$  long,  $2-3 \mu$  diameter. On leaves of *Staphylea trifolia*. Collected at Ferndale, Bucks Co., Pa., in 1933.

## 14. PHYLLOSTICTA STEIRONEMATIS Dearn. &amp; House.

On living leaves of *Steironema ciliata*. Driftwood, Cameron Co., Pa., July 14, 1933. First described by Dearness & House from New York in 1916.

## 15. PHYLLOSTICTA TRILLII Ellis &amp; Ev.

On living leaves of *Trillium* sp. Slippery Rock, Pa., June, 1933. Originally described from Pullman, Wash. Reported once from New York state. Spots subcircular to angular or irregular, dark-brown surrounded by yellow discolored leaf tissue, rather indefinite, 4-10 mm. broad; pycnidia scattered, not abundant,  $80-100 \mu$  diameter; spores cylindric, straight or curved,  $12-15 \times 2 \mu$ .

## 16. RAMULARIA AGOSERIDIS Ellis &amp; Ev.

On living leaves of *Agoseris* sp. Yellowstone Park, Wyo., July 21, 1926. Conidia  $20-24 \times 4-5 \mu$ .

## 17. RAMULARIA CILINODES J. J. Davis.

On living leaves of *Polygonum cilinodes* in company with a *Septoria*. Allegheny Nat. Forest, Forest Co., Pa., June 29, 1933. Conidia hyaline, 4-celled,  $20-44 \times 2.5-4 \mu$ . Dr. Davis indicates that the spots are caused by the *Septoria*, and *Septoria* pycnidia are present on some spots not inhabited by the *Ramularia*. Some of the conidiophores are characteristic in becoming decumbent after emerging from the stomata, and then sending out short erect branches, one from each cell, on each of which a single spore is produced. The *Ramularia* fruits are hypophyllous and the *Septoria* pycnidia are mainly epiphyllous.

18. *RAMULARIA GERANII* (West) Fuckel.

On living leaves of *Geranium viscosissimum*. Yellowstone Park, Wyo., July 22, 1926. Conidia  $12-24 \times 4 \mu$ .

19. *RAMULARIA HAMAMELIDIS* Peck.

Collected at Parrish, Forest Co., Pa., June 30, 1933, on *Hamelis virginiana*.

Spots angular, limited by the veinlets, dark purplish red above, more brownish below, 3-4 mm. long and broad, scarcely confluent; conidiophores fasciculate, the clusters appearing as numerous minute dark points almost invisible to the unaided eye, hypophyllous, 20-75 per cluster, the individuals very pale colored,  $40-80 \times 2-3 \mu$ ; spores cylindric, hyaline or nearly so,  $20-32 \times 2 \mu$ , usually 2-celled, straight.

Apparently this is also *Cercospora Hamamelidis* Ellis & Ev., a nomen nudum.

20. *RAMULARIA MIMULI* Ellis & Kell.

On languishing leaves of *Mimulus Levisii*. Yellowstone Park, Wyo., July 19, 1926. Conidia  $40-96 \times 4-5 \mu$ , 1-3-celled, hyaline. *Cercospora Mimuli* is described as producing purple-margined spots 1-2 mm. diameter, and conidia  $40-60 \times 2.5-3 \mu$ . In this collection the spots are 4-6 mm. diameter, not margined, both conidia and conidiophores are colorless, and the spores overrun the measurements for either species.

21. *SEPTOGLOEUM RHOPALOIDEUM* Dearn. & Bisby.

On living leaves of *Populus tremuloides*. Jackson Lake, Wyo., July 25, 1926. Otherwise the species is apparently known only from Manitoba. The spores in my material are somewhat smaller than the description calls for, measuring  $28-44 \times 6-9 \mu$ . They bear considerable resemblance to those of *Marssonina Populi* but are much larger and 3-celled.

22. *SEPTORIA SYMPHORICARPI* Ellis & Ev.

On living leaves of *Symphoricarpos* sp. Yellowstone Park, Wyo., July 21, 1926. Conidia  $30-40 \times 2-3 \mu$ . Originally described from North Dakota.

23. *SPHAEROPSIS SALICIS* Ellis & Barth.

On dead twigs of *Salix*, Center Co., Pa., May 12, 1935. Conidia  $18-22 \times 8-9 \mu$ . Originally described from Kansas.

24. *VERMICULARIA COPTINA* Peck.

On living leaves of *Coptis trifolia*. Collected near Cranesville, W. Va., in 1933. Spores  $12-18 \times 3-5$ , 1-celled, hyaline. Setae are absent from many of the acervuli.

## ASCOMYCETES

25. *CENANGIUM FULVITINGENS* Berk. & Curt.

Apothecia caespitose in clusters of 2 to 12, 2-4 mm. diameter, erumpent, narrowed to a short stalk-like base, externally a dark tobacco-brown or dark coffee-brown, somewhat furfuraceous, though appearing practically glabrous under a lens; hymenial disk concolorous or with a slight olivaceous tint; tissue discolored by KOH solution a dark red-brown; asci clavate, long-stalked,  $35-45 \times 4-5 \mu$ , 8-spored; spores biseriate, sub-allantoid to oblong, smooth, hyaline,  $5-6 \times 1.5-2 \mu$ ; paraphyses linear, simple, not forming a compact epithecium,  $1.5 \mu$  diameter.

On bark of dead *Acer*, Gray's Run, Lycoming Co., Pa., Sept., 1925.

Originally described from Michener's collection in Pennsylvania, this species has remained apparently unreported in the literature. Miss Cash has compared my collection with specimens, presumably the type collection, in the Michener Herbarium, and says they are the same.

## BASIDIOMYCETES

26. *Coniophora corticola* sp. nov.

Effusus, membranaceus, separabilis, olivaceo-alutaceo, ad marginem albidus; contextu  $200-300 \mu$  crasso, ex hyphis hyalinis, nodoso-septatis,  $2-3 \mu$  diam.; sporis globosis, levibus, pallide coloratis,  $6-8 \mu$  diam.; hymenio cystidiis destituto.

Ad corticem coniferarum (*Tsuga canadensis*), Charter Oak, Huntingdon Co., Pa.

Effused for several centimeters, separable as a thin delicate membrane with a broad white fimbriate margin; hymenial surface at first floccose-granulose or pitted, then forming a continuous

membranous layer, at first white, then "olive-buff," finally oliveaceous "cream-buff"; in section 200-300  $\mu$  thick, somewhat colored in the hymenial layer, otherwise colorless, composed of very loosely interwoven clamped hyphae 2-3  $\mu$  diameter bearing a few scattered crystals or resinous masses up to 10  $\mu$  diameter that are not dissolved in either KOH or lactic acid; spores globose, smooth, faintly brownish, 6-8  $\mu$  diameter, very granular when treated with glycerine; basidia with long slender sterigmata 8-10  $\mu$  long, apparently frequently 1- or 2-spored; cystidia none.

On old hemlock (*Tsuga canadensis*) bark in pile in deep woods. Type collected at Charter Oak, Huntingdon Co., Pa., March 17, 1934. (Overholts Herb. 17036.)

Externally this has much the appearance of *Coniophora polyporoidea* but the spores are entirely different. I have examined two collections of that species determined by Burt and find both in agreement in having ellipsoid or elongate-ovoid spores that are distinctly echinulate. Neither of these characters are sufficiently emphasized by Burt, but the echinulations are quite distinct, and since there is some color in the spore wall, that species belongs in *Hypochnus* rather than in *Coniophora*. I, therefore, recombine it as ***Hypochnus polyporoideus*** (Berk. & Curt.) comb. nov.

The distinctive features of the species are the creamy avellaneous color of the mature hymenium, the broad white fimbriate margin, the globose, smooth, slightly colored spores and the hyphae of small diameter, bearing clamps and scattered resinous or crystalline bodies.

**27. *Corticium albostramineum* (Bres.) comb. nov.**

I have recently had occasion to examine the types of *Peniophora albostraminea* Bres., collected by Weir in Idaho, and loaned me by John Stevenson from the mycological collections of the Bureau of Plant Industry. Bresadola reported the presence of "cystidia" and Burt expressed doubt that these organs were more than the tips of projecting gloecystidia. After examining the types it is very evident that we have here the rather unusual situation of gloecystidia with strongly projecting tips, extending as much as 40  $\mu$  beyond the surface of the basidial layer. They are quite numerous and can be traced in many instances through the thickness of the entire subiculum as well. On the immersed portion

they show better their gloeocystidial characters. Hence this species belongs in *Corticium* rather than in *Peniophora*, or if one follows the more recent segregation, the name would become *Gloeocystidium albostramineum*. On the basis of descriptions given by Patouillard, Bresadola, and Burt, *Peniophora tenuis* (Pat.) Masee, differs in having true cystidia (also gloeocystidia, fide Burt) and considerably larger spores. However, the two may represent the same species.

28. *ENTYLOMA COMPOSITARUM* Farl.

An *Entyloma* with globose spores 11–15  $\mu$  diameter, and without conidia was collected on *Agoseris purpureus* at the U. S. Gold Corporation above Eldora, Colo., elevation nearly 10,000 feet, July 31, 1926. This is an unrecorded host for any *Entyloma*. The species might be *E. polysporum* as indicated by the lack of conidia.

29. *PANUS OPERCULATUS* Berk. & Curt.—A correction (FIG. 1).

In a previous series of notes (Mycologia 25: 427. 1933) I described under this name a species that now seems referable to *P. salicinus* Peck. In that description comment was made that my specimens showed no trace of the veil called for in earlier descriptions of *P. operculatus*. Otherwise the agreement seemed to be close enough, particularly as I had examined no. 2010 of Ellis and Everhart North American Fungi (Missouri Botanical Garden Herbarium copy) and found no evidence of a veil in that collection. However, Dr. A. H. Smith with whom I have corresponded on this subject, writes me that in the University of Michigan copy of that issue there is a distinct veil present. It is conceivable that the distribution effected by Ellis and Everhart may include two species; or it may be possible that the veil fragments had disappeared in the Garden copy of that set. My notes on the internal structure of the specimens in that copy agree with my findings in the specimens previously referred to *P. operculatus*, and since this structure is different from that which I am about to describe for what I now take to be the true *P. operculatus*, I am inclined to accept the second alternative.

In the summer of 1935, while collecting at Dr. H. S. Jackson's



Bear Island Laboratory in the Temagami Forest Reserve, a specimen was brought in by Dr. R. F. Cain in which the veil was a prominent structure (FIG. 1). Although in general appearance these specimens were much like those I had previously described as *P. operculatus*, I realized that possibly, after all *P. operculatus* should have a veil, and that here probably was the true *P. operculatus*. Subsequent study showed that such was in all probability

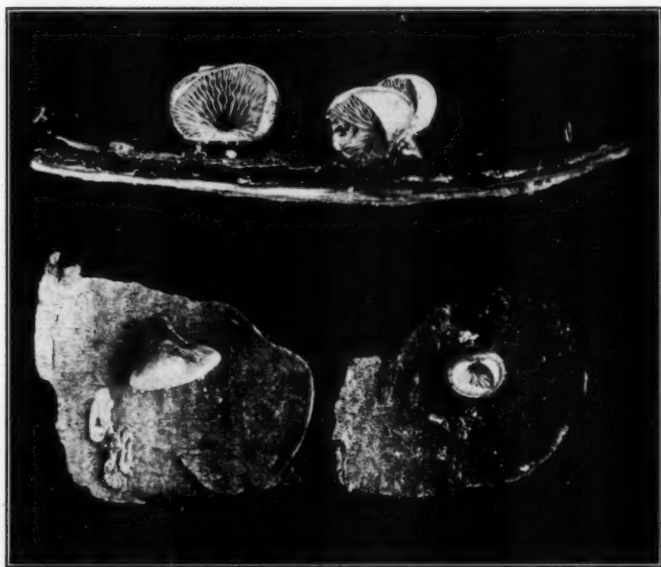


FIG. 1. *Panus operculatus*.

the case. At this point I wrote Dr. Smith for assistance, suspecting that there might be something among Dr. Kauffman's collections that would aid in a solution. He reported first, that no. 2010 referred to above does have a veil in their copy; second, that a collection in their herbarium from Michigan seems to correspond exactly to the velate specimens collected by Dr. Cain, and this was determined by Kauffman (probably on the basis of the treatment in the North American Flora) as *Panus patellaris* Fries, which Murrill listed as a synonym for *P. operculatus*. Then the question arose as to whether these two species are synonymous,

and if not how they differ. Reference to the illustration in Fries' *Icones* (pl. 176) shows a plant that, except for the veil, might cover either the velate or the evelate form. But since Fries neither described nor illustrated a veil in connection with *P. patellaris*, it would be a logical assumption, since both forms exist, that he was dealing with an evelate species. In two recent treatments of the European species of *Panus* and *Pleurotus*, respectively, Malkovsky (Ann. Myc. 30: 10-80. 1932) and Pilat (Atlas Champ. Europe 1935) do not accept this view, but treat these two species as synonymous, accepting *P. patellaris* as the earlier name, and describing it as possessing a veil. At any rate, so far as the American velate plants are concerned, they can be referred with certainty to *P. operculatus*, which may be a synonym for *P. patellaris*.

Specimens of the velate form were sent to Dr. Pilat who identified them as *P. patellaris* with *P. operculatus* a synonym. At the same time specimens of the evelate species were sent him. He would refer them to *Pleurotus* (*Panus*) *violaceofulvus* var. *Delastri* which previously I had disregarded because of the larger spores universally recorded for that species and variety. *P. salicinus* Peck is usually given as a synonym in this connection, and if the spores are constantly smaller than in the European plants it might be well to use Peck's name. That species is now represented in my herbarium by two collections from Ontario, one from New York, and four from Pennsylvania, on *Alnus* and on *Betula*. The presence of a veil is not the only character that can be used to distinguish *P. operculatus* from the evelate type. In the former species the pileus trama has a gelatinous layer 300-600  $\mu$  broad beneath the cuticle, making up about half of the thickness of the section. Such a layer is entirely lacking in the evelate form.

I append a descriptive account of the velate form which I shall now designate as *P. operculatus*.

Sporophore coriaceous, reviving well when moistened, laterally or dorsally substipitate or short-stipitate, circular to reniform, attenuate behind or dorsally to a substipitate base, dry or slightly viscid, apparently at first somewhat furfuraceous-flocculose, soon glabrous, brown or dark brown, drying firm and hard, 0.5-2 cm. long, 0.8-2.5 cm. broad; margin strongly incurved, even; context

pallid; gills pale brown, close, radiating from an excentric point, 1-2 mm. broad; stem a lateral or dorsal prolongation of the pileus, 3-4 mm. thick, rather distinct but only 3-4 mm. long where best developed, concolorous with pileus; spores allantoid, smooth, hyaline,  $4.5-6 \times 1-1.5 \mu$ ; cystidia present on and near edges of gills, not abundant, cylindric or slightly inflated below, projecting up to  $30 \mu$ ,  $4-5 \mu$  diameter; trama of pileus showing three rather distinct regions in sections—(1) a cuticular covering rather dark in color and  $100-150 \mu$  thick, (2) a gelatinous middle layer  $350-600 \mu$  thick, and (3) an ungelatinized layer from which the gills arise,  $900-1200 \mu$  thick; gills covered by a distinct white veil which finally splits and leaves fragments on the margin of the pileus.

On dead *Alnus*, erumpent from the bark. Gregarious. A collection from Bear Island, Lake Temagami, Ontario, and one from Sault St. Marie, Mich. (collected Baxter, communicated Smith), have been examined.

### 30. STEREUM DURIUSCULUM Bres.

Resupinate except for an elevated black tumid margin, expanded for several centimeters, hard and firm, 1-4 mm. thick, in section showing a black substratal layer and a brown and much thicker context layer; hymenial surface pallid to avellaneous, somewhat silky under a lens; in section brown throughout, layered in 6 to 7 layers that are darker and broader toward the substratum and everywhere composed of pale brown interwoven hyphae, much branched, stiff, ending in short prong-like or spiculate tips about  $2 \mu$  diameter; basidial layer scarcely evident, usually of isolated basidia among spiculosely branched hyphae tips; spores globose, hyaline,  $5-7 \mu$  diameter, passed as smooth in KOH mounts but in lactic acid distinctly asperulate; cystidia and gloecystidia apparently lacking; no paraphyses; imbedded spores through the subiculum duplicate the characters of the basidiospores.

On dead wood of deciduous trees. A single collection has been received from Dr. Hesler, collected in Tennessee.

In consistency and thickness this species approaches *S. Murrayi*, *S. sulcatum*, *S. subpileatum*, and others, but is entirely lacking in cystidia and vesicular structures and the antlered branching of the stiff hyphae is totally at variance with species of that group. It is placed in *Asterostromella* by Bourdot & Galzin. This is the first record of the occurrence of this species in America.

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## NOTES ON SOME USTILAGINALES FROM INDIA

G. P. CLINTON AND GEORGE L. ZUNDEL

The species of Ustilaginales or smuts reported in this paper were collected in India by R. R. and I. D. Stewart. Submitted to the senior author by the collectors for identification, the determination of species was made during the winter of 1926-1927 at New Haven. Among the fungi listed below are certain species that have been apparently hitherto unreported from India, as well as certain new host plants for some of the species of smuts.

USTILAGO HORDEI (Pers.) Kell. & Sw. Ann. Rep. Kan. Exp. Sta. 2: 268. 1890.

On *Hordeum* sp. (cult. barley), Khangah Dogran, Gujranwala District, March 11, 1917 (Punjab Plants 1445); Nadi, Dharm-sala; Stewart (Plants of North West Himalaya 2016).

USTILAGO STRIAEFORMIS (West.) Niessl, Hedwigia 15: 1. 1876.

On undetermined grass, Sonamarg, Kashmir, Aug. 30, 1921 (Plants of North West Himalaya 6857).

USTILAGO TRITICI (Pers.) Rostr. Overs. Danske, Vid. Selsk. Forh. 1890: 15. Mr 1890.

On *Triticum* sp. (cult. wheat), Khangah Dogran, Gujranwala District, March 11, 1917 (Punjab Plants 1446).

USTILAGO UTRICULOSA (Nees.) Tul. Ann. Sci. Nat. III. 7: 102. 1847.

On *Polygonum* sp.; Kanga-Gund, Kashmir, elev. 6000 ft., September 7, 1922 (Plants of North West Himalaya 7536).

On *Polygonum* sp.; Pahlgam, 7300 ft. elev., September 4, 1930 (Plants of Kashmir 5891).

On *Andropogon annulatus* (*A. Bladhii*); Pathankot, Gurchas-

pur District, 1000 ft. elev., May 11, 1917 (Plants of the Punjab 1776).

*SPHACELOTHECA HYDROPIPERIS* (Schum.) deBary, Vergl. Morph. Pilze 187. 1884.

On *Polygonum* sp.; Grinagar, 5500 ft. elev., September 7, 1922 (Plants of Kashmir 7500½ and 7536).

*SPHACELOTHECA PANICI-MILIACEI* (Pers.) Bubak, Naturw. Landes. Böhmen 15: 26. 1916.

On *Panicum miliaceum*; Kangan, Scinde Valley, 6000 ft. elev., September 7, 1917 (Plants of Kashmir 3638).

*SPHACELOTHECA SCHOENANTHI* (H. & P. Sydow & Butler) Zundel, Mycologia 22: 136. 1930.

On *Cymbopogon confertiflorus*; 15th mile Dalhousie Road, February 14, 1917 (Punjab Plants 1168).

*SOROSPORIUM REILIANANUM* (Kuhn) McAlpine, Smuts Austr. 181. 1910.

On *Zea Mays*; Sonamarg, Kashmir, September 7, 1917 (Plants of North West Himalaya 3750).

*CINTRACTIA CARICIS* (Pers.) Magnus, Abh. Bot. Ver. Brand. 37: 79. 1896.

On *Carex* sp.; Sonamarg, Kashmir, 12,000 ft. elev., August 30, 1921 (Plants of Kashmir 6847); also Sonamarg, Kashmir, July 23, 1921 (Plants of North West Himalaya 6409).

*UROCYSTIS MAGICA* Pass.; Thüm. Myc. Univ. 223. 1875.

On *Allium rubellum*; Rawalpindi, elev. 1700 ft., March 8, 1922 (Plants of the Punjab 6950½).

*UROCYSTIS STIPAE* McAlpine, Smuts Austr. 198. 1910.

On *Stipa sibirica*; Sonamarg to Baltal, August 20, 1921 (Plants of Kashmir 6705, 3751).

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# THE *CERCOSPORA* LEAF SPOT OF ROSE CAUSED BY *MYCOSPHAERELLA* *ROSICOLA*<sup>1</sup>

B. H. DAVIS<sup>2</sup>

(WITH 7 FIGURES)

The *Cercospora* leaf spot of rose has long been known and the conidial stage of the pathogene, *Cercospora rosicola* Passerini, has been collected frequently in many parts of the world. In spite of this, our knowledge of the disease and the pathogene is very incomplete. In the summer of 1932 the writer noted that a number of species of *Rosa* in the rose gardens of the Department of Floriculture and Ornamental Horticulture at Cornell University, Ithaca, N. Y. were almost entirely defoliated by the pathogene. At that time a study of the disease and pathogene was undertaken. The results of the investigation, together with a review of the studies made by other workers, are reported herein.

The disease occurring on roses about Ithaca, N. Y. is caused by *Mycosphaerella rosicola* (Pass.) comb. nov. The conidial stage, *Cercospora rosicola* Pass., is the common species of *Cercospora* on rose in the United States.

## SUSCEPTS

As far as is known only the rose is affected by this disease. Inoculations made on red raspberry using conidia of *Mycosphae-*

<sup>1</sup> A portion of a thesis presented to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup> The writer wishes to express his appreciation of the helpful criticism and encouragement of Dr. L. M. Massey, under whose direction this work has been done. He is indebted to Dr. H. M. Fitzpatrick for help in connection with the mycological aspects of the studies and to Dr. Charles Chupp who because of his intimate knowledge of the genus *Cercospora* was in a peculiarly favorable position to help him in the identification of the species of *Cercospora*. Thanks are due also to Dr. D. H. Linder, of Harvard University and Dr. F. J. Seaver, of the New York Botanical Garden, for their kindness in loaning specimens of type material.

*rella rosicola* failed to give infection. The following species<sup>3</sup> have been reported to be affected or have been mentioned on specimen packets in herbaria: *Rosa blanda* Ait., *R. setigera* Michx., *R. nitida* Willd., *R. californica* Cham. & Schlecht, *R. centifolia* Linn., *R. chinensis* var. *semperflorens* Koehne and *R. carolina* Linn. Besides these the writer has found the disease occurring on the following: *R. pisocarpa* Gray, *R. gymnocarpa* Nutt., *R. virginiana* var. *alba* Willmott, *R. Woodsii* Lindl., *R. Woodsii* var. *Fendleri* Rydb. and *R. multiflora* Thunb.

The following varieties of rose have been reported to be affected: Baltimore Belle and Queen of the Prairies (15). The writer has found the disease occurring on Souvenir D'Ernest Thebault and André Louis.

#### THE DISEASE

NAME. In the literature this disease has been called "leaf spot" (13), "leaf-spot of the rose" (15) (7), and "*Cercospora* rose-leaf spot" (16). It is referred to here as "*Cercospora* leaf spot of rose."

HISTORY AND RANGE. The first collection of diseased specimens was made in 1874 by Passerini. Since then the disease has been observed in many parts of the world. At the present time it is known to occur in Europe (14) (9), Siberia (14) (9), India (17), Australia (7), the Philippines (16), Porto Rico (19), Trinidad (13), North America (5), and South America (2).

The first known collection of diseased leaves in the United States was made in Florida in 1882. Part of the material is in the Ellis Herbarium. In 1885 Ellis and Everhart (5) recorded the pathogene on rose in their publication on the *Cercosporae* of North America. Since then the disease has been observed in various parts of the country. It is very likely that the disease occurs throughout the range of the rose.

In 1933 Grieve (7), in a discussion of this disease, gave some of the susceptes and symptoms and a description of the pathogene. Very little work has been done on the disease.

IMPORTANCE. There are no data on the economic losses due to the disease. It does not compare in destructiveness with black

<sup>3</sup> The scientific names of the susceptes are taken from The Standard Cyclopedia of Horticulture, 1922, by L. H. Bailey.

spot and brown canker but attacked plants may be as severely injured as those affected by anthracnose. Lesions do not occur on the stems, as in the case of anthracnose, but defoliation is much more severe. By the middle of August the species of rose under observation at Ithaca are practically defoliated. Plants are not killed but early defoliation year after year probably brings about a weakened condition. In addition to this the plants become unsightly rather early in the season.

#### SYMPTOMATOLOGY.

**Morphologic symptoms.** The disease has been found only on the leaves. Inoculations on young vigorous growing branches gave many infections on the leaves but none on the stems. Many spots may develop on a single leaflet and may occur on any part of the leaf including the petioles, midribs, and stipules. Spots are circular in shape and have a definite margin. Single spots may reach 10 mm. in diameter, the usual size being 2 to 4 mm. The size of the spot varies considerably with the species and variety affected.

The first symptom is a small purplish area. As the disease progresses a small necrotic area appears and gradually enlarges. On the upper surface of the leaf the necrotic area is buffy <sup>4</sup> brown to cinnamon brown in color. About the necrotic area there is a narrow border which ranges in color from taupe brown to raisin black. Sometimes there is a narrow zone of a purplish color surrounding this border. On the under side of the leaf the necrotic area ranges in color from citrine drab to cinnamon brown. The border, when present, is of a faint purplish color. When leaves become heavily infected defoliation occurs. Diseased leaves of *R. Woodsii* var. *Fendleri* are shown in figure 1.

**Histologic symptoms.** Sections of diseased leaves show the mycelium of the fungus ramifying intercellularly. The mycelium, which is rather meager, is found in the palisade and mesophyll tissues and extends as far as the purple border. The cells at the outer edge of the spot gradually lose their chlorophyll and develop a purplish pigment in the protoplasts. Those nearer the necrotic center show only the purple pigment while the protoplasts show evidence of disintegration. The cells at the margin between

<sup>4</sup> Names of colors are according to *Color Standards and Color Nomenclature* by Robert Ridgway (1912).



the necrotic center and the purple border gradually lose the purple pigment and the protoplasts gradually turn brown and die. In the necrotic area the brown protoplasts shrink very little. The walls do not collapse with drying but remain for the most part attached to adjoining cell walls.

**Signs.** The signs of the disease are not very striking to the naked eye, but under a hand lens small tufts of conidiophores can be seen grouped together to form a black dot (FIG. 1) or scattered over the necrotic area of the lesion. They arise from small dark brown stromata. These tufts of conidiophores help in diagnosing this disease since the spots are somewhat similar to those caused by *Sphaceloma Rosarum* (Pass.) Jenkins and *Cryptosporella umbrina* (Jenkins) Jenkins and Wehmeyer.

#### ETIOLOGY.

In identifying the species of *Cercospora* collected by different workers and deposited in herbaria the writer has found that the distinction between the several species described in the literature is not clear. Although von Höhnelt (8) in his study of *Cercospora Rosae* (Fuckel) von Höhnelt clarified the situation in regard to that species, a comparative study of the *Cercosporae* on rose has not been made. Examining all the specimens in the Cornell Herbarium and such type material as could be obtained elsewhere, the writer has made a comparative study.

Although in the literature ten names have been applied to *Cercosporae* on rose, study shows that there are only three distinct species. These are *Cercosporae Rosae* (Fuckel) von Höhnelt, *Cercospora rosicola* Passerini and *Cercospora hyalina* Muller and Chupp. The synonymy is given below:

*C. Rosae* (Fuckel) von Höhnelt (1903)

= { *Exosporium Rosae* Fuckel (1869)  
*C. hypophylla* Cavara (1899)  
*C. Rosae-alpinae* Massalongi (1900)

*C. rosicola* Passerini (1875)

= { *C. rosigena* Tharp (1917)  
*C. rosicola* var. *undosa* J. J. Davis (1922)  
*C. Rosae* Van Hook (1928)  
*C. Rosae-indianensis* Van Hook (1929)

*C. hyalina* Muller and Chupp (1934)

In 1903 von Höhnelt reported a species of *Cercospora* on *R. pendulina* as different from *C. rosicola* Passerini and specifically identical with *Exosporium Rosae* Fuckel. Recognizing that the species named by Fuckel (6) is not an *Exosporium*, von Höhnelt

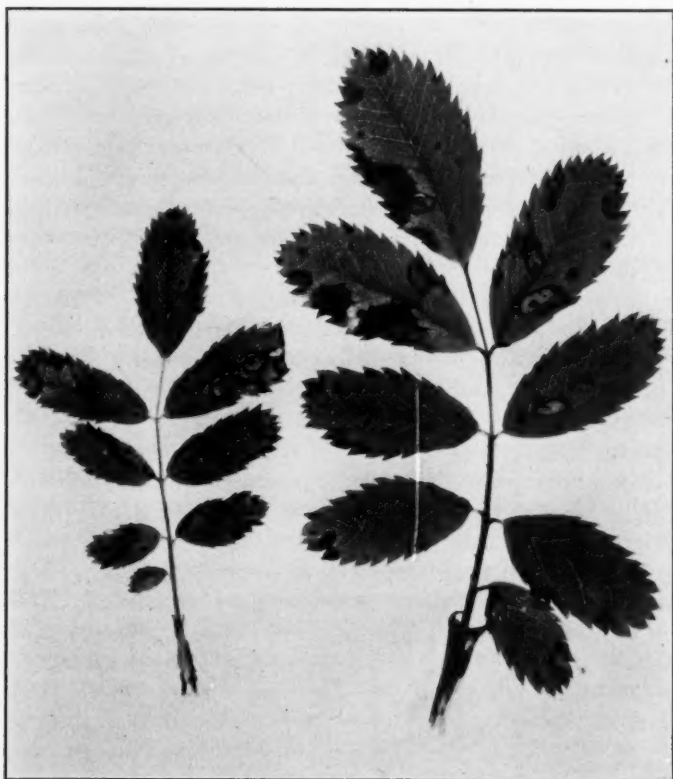


FIG. 1. Lesions on *R. Woodsii* var. *Fendleri* caused by *Mycosphaerella rosicola*. Natural infection.

amended the description and used the new combination *C. Rosae* (Fuckel) von Höhnelt. He listed as synonyms *C. hypophylla* Cavara (3), *C. Rosae-alpinae* Massalongo (10), and *C. rosicola* Allescher & Schnabl (1).

Comparison of the type material of *Exosporium Rosae* Fuckel

(Fungi Rhenani No. 1658) and specimens of *Cercospora Rosae* in von Höhnel's herbarium and in Sydow's Mycotheca Germanica (No. 2040) shows them to be specifically identical. Although specimens of *C. hypophylla* and *C. Rosae-alpinae* have not been examined it is clear from the original descriptions that they are the same as *C. Rosae*. Doctor Charles Chupp of Cornell University has very kindly examined No. 498 of Allescher and Schnabl's Fungi Bavarici in the herbarium of the Bureau of Plant Industry at Washington, D. C. and reports that this specimen, which was distributed under the name *C. rosicola* Passerini, is in fact also specifically identical with *C. Rosae*. Therefore it represents merely an incorrect identification by Allescher and Schnabl. von Höhnel, then, is not justified in citing it as a synonym of *C. Rosae*.

It is possible to distinguish *C. Rosae* from *C. rosicola* by its hyaline to olivaceous stroma, by its dense fascicles of short and almost hyaline conidiophores, and by its cylindrical conidia (FIG. 2). A description of *C. Rosae* follows:

*C. Rosae* (Fuckel) von Höhnel

Spots large, irregular in shape, brown, with little or no purplish border; fructification hypophyllous, stroma prominent, hyaline to olivaceous, 21 to 65  $\mu$  in diameter (von Höhnel 30–120  $\mu$ ); fascicles dense; conidiophores subhyaline, not geniculate, short, 15–36  $\mu$  (von Höhnel 8–24  $\mu$ )  $\times$  2.6–4  $\mu$ ; conidia cylindrical with truncate base and faintly yellowish tinged, continuous to 1-septate, 20–45  $\mu$  (von Höhnel 35–55  $\mu$ )  $\times$  2.5–4  $\mu$ .

*Synonymy:* *Exosporium Rosae* Fuckel, *C. hypophylla* Cavara, *C. Rosae-alpinae* Massalongo.

Occurring in Europe. Unknown in America.

There are conflicting statements in the literature with regard to the location of the type material of *C. rosicola*. According to an anonymous writer who gave a description of this species in Just's Botanischer Jahresbericht (3: 276. (1875) 1877), the type occurs in von Thümen's Mycotheca Universalis No. 333. This is stated to be true by Saccardo (14) also. Lindau (9) cites instead von Thümen's Herbarium Mycologicum Oeconomicum No. 333. Since examination has revealed that No. 333 of Herbarium

Mycologicum Oeconomicum is *C. rosicola* and No. 333 of Mycotheca Universalis is not a *Cercospora*, the former is accepted by us as the type specimen. The mistake made by the anonymous writer is apparently merely one of citation. Herbarium Mycologicum Oeconomicum No. 333 bears the original description, was collected by Passerini in 1874, and was distributed in 1875.

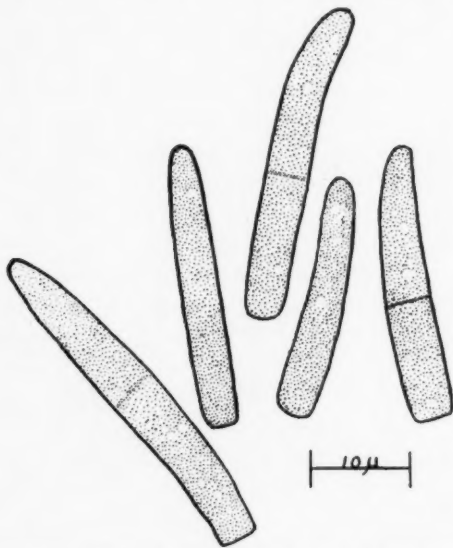


FIG. 2. *Cercospora Rosae*. Spores from Sydow's Mycotheca Germanica No. 2040. Drawn with aid of camera lucida.

Another specimen of the species occurs in von Thümen's Mycotheca Universalis as No. 1086. This packet, which was distributed in 1878, also bears the name *C. rosicola* Passerini nov. spec. and a description. Since this material also was collected by Passerini in 1874, it is likely that it is a part of the type collection. It has been examined by the writer. Doctor Chupp has examined both this specimen and No. 333 of Herbarium Mycologicum Oeconomicum and according to him they are the same.

The common species in the United States has been found to be *C. rosicola*. Its distinguishing characteristics are: stroma brown, inconspicuous, conidiophores in loose fascicles, brown, long, geniculate; conidia olivaceous, wide (averaging  $4\mu$  or more).

An examination has been made of many specimens of *C. rosicola* on several species and varieties of *Rosa* from various parts of North America. All these specimens agree on such essential points as the type of stroma and fascicles, color and geniculation of conidiophores, and color and shape of conidia. However, they vary in the length of the conidia and conidiophores and in the width and septation of the conidia. It has been found that under greenhouse conditions the conidiophores are often longer than those produced on the same species in nature. It is apparent that this species varies with the susceptible on which it grows and with environmental conditions.

Type material of *C. Rosae-indianensis* Van Hook was examined. This was first called *C. Rosae* by Van Hook (20). Finding that this name had been used previously by von Höhnelt, Van Hook (21) gave it the new name *C. Rosae-indianensis*. It appears to the writer that the "much longer conidiophores and spores" on which Van Hook differentiated his species are only variations equivalent to those found by us among typical conidiophores and spores of *C. rosicola*.

Specimen No. 3412 of Fungi Columbiani was examined and the writer believes that the "long slender more or less spreading and undulate conidiophores" on which Davis (4) erected *C. rosicola* var. *undosa* represent also only such variation as one may find among typical conidiophores of *C. rosicola*.

Doctor Chupp examined type material of *C. rosigena* Tharp (18) in the herbarium of the Bureau of Plant Industry at Washington, D. C. and found it specifically identical with *C. rosicola*. A description of *C. rosicola* follows:

*C. rosicola* Passerini

Spots uniformly reddish brown or purplish, with or without a light brown to tan center, 2-10 mm. in diameter, circular; fructification amphigenous, mostly epiphyllous; stromata inconspicuous, scattered over the necrotic area or grouped together, brown; conidiophores in loose fascicles, brown, strongly geniculate, typically continuous but sometimes one- to two-septate, 45-120 (Davis, as long as 150  $\mu$ )  $\times$  4.6-6  $\mu$ , usually 50-60  $\times$  5  $\mu$ ; conidia obclavate, with a beveled base, olivaceous, 1-6-septate, straight or slightly curved, 30-75  $\times$  3.5-5.5  $\mu$ , usually 40-60  $\times$  4-5  $\mu$  (FIG. 3).

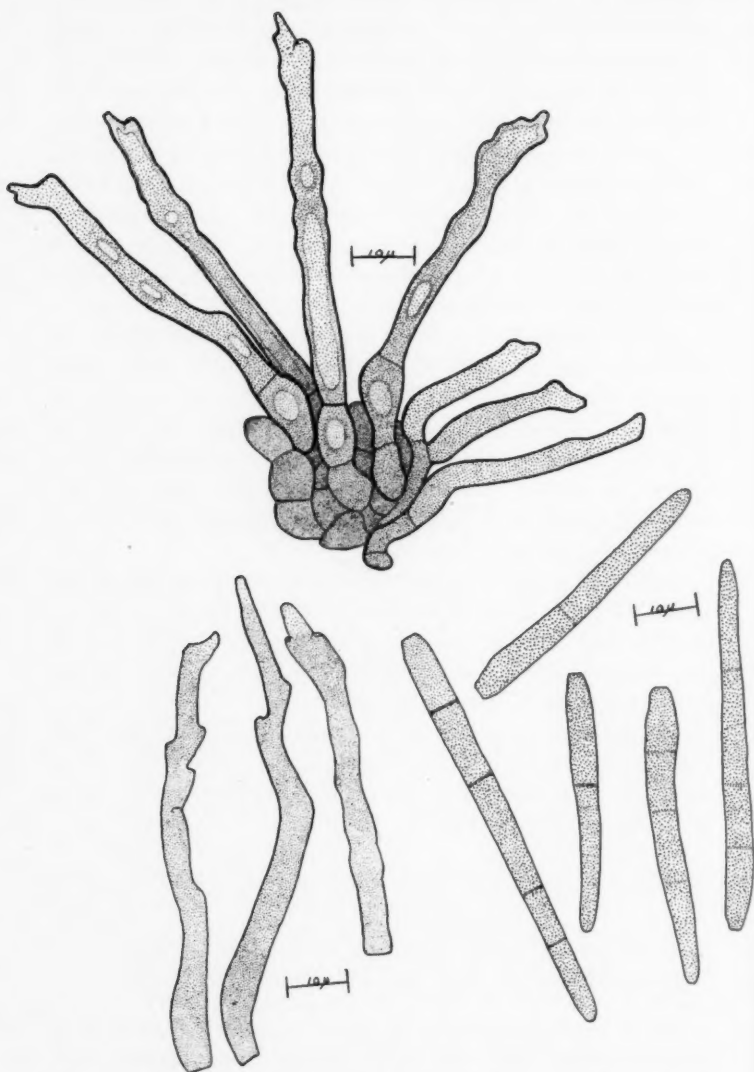


FIG. 3. *Cercospora rosicola*. Stroma and conidiophores, conidiophores and conidia. Drawn with aid of camera lucida.

Occurring in Europe, Australia, North America and South America.

Though the original spelling of the specific name of Passerini's species was "*rosaecola*," Saccardo changed the spelling to "*rosicola*" thus giving it the correct Latin form and spelling. We have therefore accepted the change made by Saccardo which is permissible under the rules of the International Code of Nomenclature.

While examining the material deposited in the herbarium at Cornell University, a specimen of a *Cercospora* was found which is specifically distinct from both of the two above discussed species. In this case (FIG. 4) the stroma is brown and prominent, the fascicles are dense, the conidiophores are short, olivaceous, and lacking in geniculations, and the conidia are long, obclavate, olivaceous and narrow (averaging less than  $3\ \mu$  in width). This specimen was collected at Savannah, Georgia, by J. Conrad Puder in 1915. Additional material of this species was found on leaves collected in Florida by R. D. Dickey. A description of this species follows:

***Cercospora Puderii* sp. nov.**

Spots brown or greyish brown with taupe brown border, circular, 2-5 mm. in diameter; fructification amphigenous; stroma prominent, brown, 18-36  $\mu$  in diameter, usually 25  $\mu$ ; fascicles dense; conidiophores olivaceous with brownish base, not geniculate, or only slightly so, continuous to 3-septate, short, 13-24  $\times$  2.6-4  $\mu$ , usually 20  $\times$  3.3  $\mu$ ; conidia obclavate with a beveled base, pale olivaceous, 1-7-septate, straight or curved, 30-75  $\times$  2.0-3.5  $\mu$ , usually 40-50  $\times$  2.6  $\mu$ .

Type material deposited in Cornell University Herbarium as No. 18220.

Known only from the southern United States.

Recently Muller and Chupp (12) described from South America a fourth species, *C. hyalina*. Its essential characteristics are indicated in the following key.

A key to the four known species of *Cercospora* on rose follows:

- A. Spores hyaline or very faintly colored, base more or less truncate.
- B. Spores more nearly obclavate than cylindrical, base conically truncate to truncate, tip somewhat acute, 2-3  $\times$  40-150  $\mu$ ; stroma slight

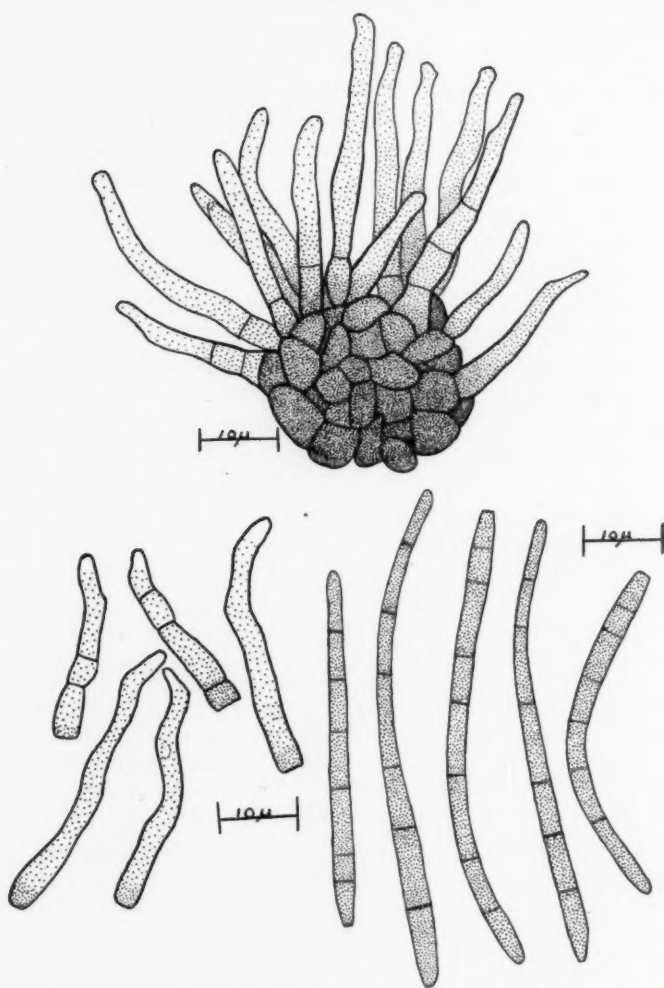


FIG. 4. *Cercospora Puderii*. Stroma and conidiophores, conidiophores, and conidia. Drawn with aid of camera lucida.



or none; fascicles usually not dense; conidiophores slightly geniculate; fruiting usually on the upper leaf surface; producing spots with minute white centers and purple borders.

*C. hyalina* Muller & Chupp

- BB. Spores more nearly cylindrical than obclavate, faint yellowish tinge, base subtruncate, tip bluntly rounded,  $2.5-4 \times 20-55 \mu$ ; stroma prominent; fascicles dense; conidiophores not geniculate; fruiting confined to lower leaf surface; producing relatively large spots or blotches without white centers or evident purple borders.

*C. Rosae* (Fuckel) v. Höhn.

- AA. Spores plainly colored, olivaceous, base usually not truncate.

- B. Spores  $3.5-5.5 \times 30-75 \mu$ ; stroma usually not prominent; fascicles usually not dense; conidiophores long, geniculate; producing spots uniformly reddish brown or purplish, with or without a light brown to tan center.....*C. rosicola* Pass.

- BB. Spores  $2-3.5 \times 30-75 \mu$ ; stroma prominent; fascicles dense; conidiophores olivaceous, comparatively short, not often geniculate; producing spots with minute white centers and reddish brown margins.....*C. Puderii* Davis

### Connection of imperfect stage with perfect stage

For the past two years leaves heavily infected with *C. rosicola* have been placed outdoors in the autumn between pieces of wire-screen. Some of the leaves examined as early as February 19 showed many immature perithecia in and about the old spots formed the previous season. These leaves were placed in a moist chamber and after a period of two weeks mature asci and ascospores were found.

In certain spots fruit-bodies which were thought to be perithecia proved, on examination, not to contain asci or ascospores. However, they showed fascicles of conidiophores of *C. rosicola* (FIG. 5). This suggested the connection of the *Cercospora* with the perithecial fungus.

Many single ascospore cultures were made in the following manner. Drops of sterile water were placed on the under side of the lid of a sterile petri dish. Bits of leaves containing perithecia were placed in these drops. Drops of sterile water were then placed in the bottom of the petri dish directly beneath the bits of leaves. When a considerable number of ascospores had been discharged a small wire loop was dipped into the drops of water and streaked across the surface of an agar plate. In 24 hours the spores had germinated. When a germinated spore was found

separated from others it was transferred to another agar plate. After a period of 10 days these cultures showed conidiophores and conidia of *C. rosicola*.

Potted plants of *R. Woodsii* var. *Fendleri* were inoculated using conidia from single ascospore cultures. Conidia were atomized



FIG. 5. Photomicrograph showing conidiophores of *Mycosphaerella rosicola* borne on a sterile perithecium on overwintered rose leaves.

on the leaves and the plants placed in a moist chamber for a period of seventy-two hours. Then they were placed in a greenhouse at a temperature around 80° F. After 10 days spots began to appear on the leaves. At the end of three weeks they were typical of those observed in nature and showed conidiophores and conidia of *C. rosicola*. The pathogene has been reisolated in cultures from these lesions.

**The ascigerous stage.** The perithecia occur rather thickly in and about the old spots formed the previous season. At maturity they are erumpent, globose, and black. The wall, which varies from 1 to 3 cells in thickness, is membranaceous. The asci,

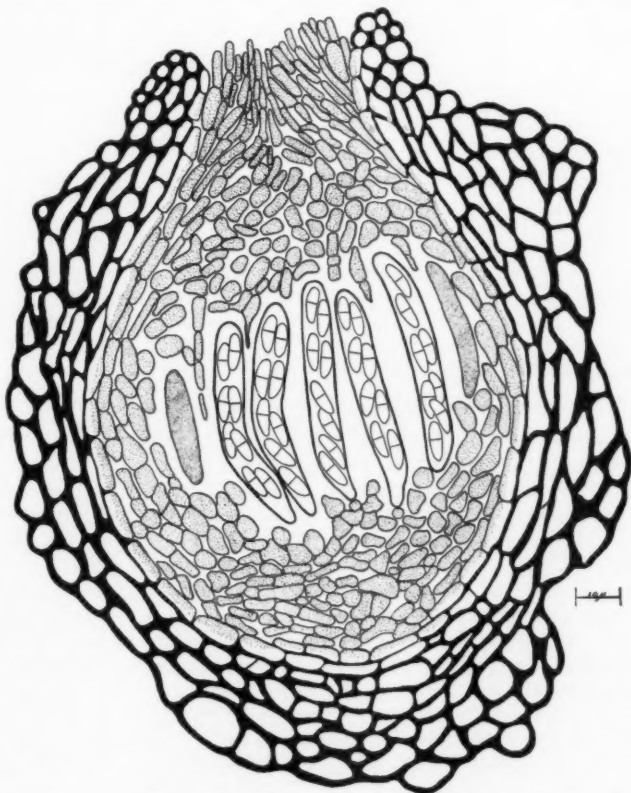


FIG. 6. Longitudinal section through a perithecium of *Mycosphaerella rosicola*. Drawn with aid of camera lucida.

borne in a definite fascicle, are clavate, astipitate, and 8-spored. The walls of the asci are thickened toward their tips. The ascospores, which are olivaceous in color, are biseriate or sub-biseriate, and unequally 2-celled with the smaller cell toward the tip of the ascus. The characters of the ascigerous stage place it in the genus

*Mycosphaerella*. A longitudinal section through a perithecium is shown in figure 6. Ascospores and immature and mature asci are shown in figure 7.

Although these fruit bodies have been called perithecia, it is apparent in the light of the investigations of Miller (11) that they are not true perithecia. Since bits of stromatic tissue can be found extending down between the asci it appears that the cavity in the stroma is formed by the dissolution of the stromatic tissue during the time that the asci are increasing in size. This supposition is also borne out by the fact that the covering about the ascigerous cavity is thicker in the early stages of the development of the asci than at maturity (FIG. 6).

A study of the fungi reported on rose reveals no ascomycete with the above characteristics. The following new combination is proposed.

***Mycosphaerella rosicola* (Pass.) comb. nov.**

Synonymy: *Cercospora rosicola* Pass., *C. rosigena* Tharp, *C. rosicola* var. *undosa* J. J. Davis, *C. Rosae* Van Hook, *C. Rosae-indianensis* Van Hook.

Perithecia amphigenous but usually hypophyllous, erumpent, black, borne singly but rather thickly, 65 to 105  $\mu$  in diameter, usually 75–80  $\mu$ ; asci astipitate, clavate, with walls slightly thickened toward the tips, 36–57  $\times$  9–11  $\mu$ , usually 45  $\times$  9  $\mu$ ; apara-physate; spores biseriate or sub-biseriate, unequally 2-celled with the smaller cell toward the apex of the ascus, not constricted at the septum, slightly curved on one side and flattened on the other, rounded on the ends, olivaceous, 13–17  $\times$  4–5.3  $\mu$ .

On overwintered leaves of *Rosa Woodsii* var. *Fendleri*. Specimens deposited in the Herbarium of Cornell University as No. 23392.

SUMMARY

In this study on the *Cercospora* leaf spot of rose, an ascigerous stage found on overwintered leaves is connected with the conidial stage, *Cercospora rosicola* Pass. As a survey of the literature reveals no ascigerous stage specifically identical with this one, the combination *Mycosphaerella rosicola* (Pass.) comb. nov. is pro-

posed. The known susceptibles are listed and the range, importance, and symptoms of the disease are given. The pathogenicity is proved by inoculation experiments.

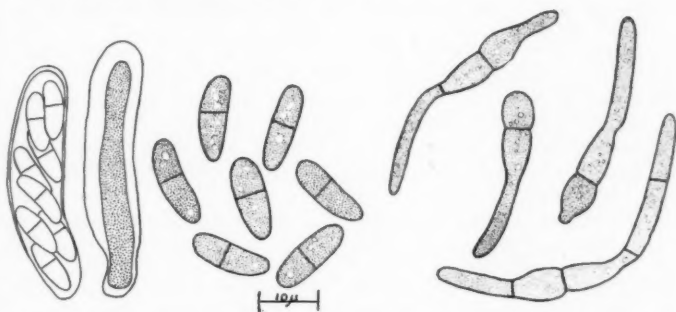


FIG. 7. *Mycosphaerella rosicola*. Immature and mature asci, ascospores, and germinated ascospores. Drawn with aid of camera lucida.

A comparative study of the *Cercosporae* described in the literature as occurring on the rose shows that there are in reality but three species. These are *C. Rosae* (Fuckel) v. Höhn., *C. rosicola* Pass. and *C. hyalina* Muller and Chupp. Descriptions and synonymy of these species are given. In the course of the study a fourth species of *Cercospora* collected in the southern United States was studied and is described as new under the name *Cercospora Puderii*.

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## PERITHECIAL MATERIAL OF ERYSIPHE AND MICROSPHAERA ON TRIFOLIUM PRATENSE

GRACE A. PETERSEN <sup>1</sup>

(WITH 4 FIGURES)

Powdery mildew of red clover, prior to 1921, was of extremely uncommon occurrence in the eastern United States. That year it suddenly became more abundant and in 1922 reached epiphytotic proportions in several states in the eastern half of the country. Massachusetts and Michigan reported it as common everywhere. Pennsylvania, Ohio, Minnesota, and Iowa reported seriously destructive outbreaks, local in some cases, statewide in others. Collectors, interested in learning the identity of the species, searched for perithecia and met with a surprising lack of success. The Plant Disease Bulletin of the United States Department of Agriculture for July 1, 1922, states: "The name of the mildew is not known definitely, for as yet the perithecial stage of the fungus does not seem to have been found. Efforts should be made to discover the perfect stage this year and thus settle the question of nomenclature."<sup>2</sup> Reporting for Pennsylvania, C. R. Orton stated that a careful search throughout the preceding season had failed to reveal perithecia. Reports from the other states in which the conidial stage was so abundant were also negative; and in the years that have followed, eastern plant pathologists have not reported finding any perithecial material.

The Plant Disease Bulletin for August 1922 stated, however, that the perfect stage of *Erysiphe Polygoni* DC. had meanwhile been reported as having been found on red clover in Washington,<sup>3</sup> Oregon, and Idaho as early as 1915; and that B. F. Dana had re-

<sup>1</sup> The writer gratefully acknowledges her indebtedness to Professor H. M. Fitzpatrick, who suggested the publication of this paper and supervised its preparation.

<sup>2</sup> Plant Disease Survey. The Plant Disease Bulletin 6: 8-14. 1922.

<sup>3</sup> The Washington material was cited as "*Erysiphe communis*."

ported it for 1922 as common in Washington.<sup>4</sup> Following the publication of these records, J. L. Sheldon of West Virginia wrote to the Plant Disease Survey that he had perithecial material of

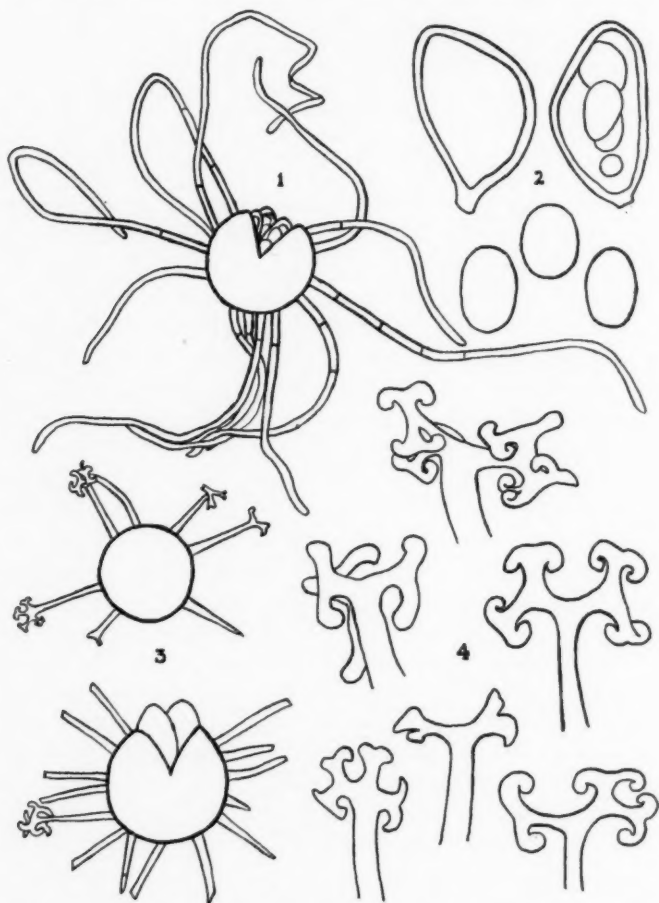


FIG. 1, perithecium of *Erysiphe Polygoni* DC.; 2, asci and spores of *Erysiphe Polygoni* DC.; 3, two perithecia of *Microsphaera Alni* (Wallr.) Salmon, showing variation in size; 4, tips of six appendages of *Microsphaera Alni* (Wallr.) Salmon.

<sup>4</sup> Plant Disease Survey. The Plant Disease Bulletin 6: 53-55. 1922.



*E. Polygoni* on *Trifolium pratense* L. collected at Morgantown, West Virginia, in 1908. No other eastern collection of the perfect stage of this fungus on red clover has been reported. The Office of Pathological Collections in Washington, D. C., has perithecial specimens of this species from several western states, but its collections from the East are of the conidial stage only.<sup>5</sup>

During the summer of 1937, the writer collected the perfect stage of *E. Polygoni* (FIG. 1, 2) on *T. pratense* from two different stations in Ithaca, New York. Material from four other stations in Ithaca bore perithecia of *Microsphaera Alni* (Wallr.) Salmon (FIG. 3, 4). Measurements of perithecia, asci, and spores corresponded in each case with those given by Salmon<sup>6</sup> for these species. Our collections have been deposited in the Plant Pathology Herbarium at Cornell University (Numbers 26836-26839, 26841, 26862). None of the leaves examined bore more than a few scattered perithecia. Their presence was not discernible without the aid of a binocular. This may account for the seeming rarity of the perfect stage here in the East. The collection of *M. Alni* seems especially interesting since, to the writer's knowledge, it has never before been reported on *Trifolium*, either in America or elsewhere.

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<sup>5</sup> This information was very kindly provided by Mr. John A. Stevenson, mycologist in charge of the Mycological Collections of the Bureau of Plant Industry.

<sup>6</sup> Salmon, E. S. A monograph of the Erysiphaceae. Mem. Torrey Club 9: 132-133, 178. 1900.

## TWO NEW OPERCULATE CHYTRIDS

J. S. KARLING

(WITH 37 FIGURES)

In the course of an examination of dying and degenerating algae collected in New Jersey during the summer of 1937, two rhizidiaceous operculate chytrids were found which are distinctive in several ways and merit consideration as new species. The first one occurred on *Spirogyra crassa* and relates to the genus *Chytridium*. It is characterized chiefly by an amber to dark brown persistent zoospore case attached basally and somewhat laterally as a slightly pointed, semicircular protuberance to the sporangium, and a pronounced gregarious association which doubtless results from the feeble motility of the swarmspores. I am accordingly naming it *C. aggregatum*. The second species which was found in dead internodes of *Chara coronata* belongs in the genus *Endochytrium* and is distinguished by one to several blunt digitations at the base of the sporangium or on the main axis of the rhizoidal system, and light to medium brown resting spores. With the view of emphasizing the former character, the name *E. digitatum* is proposed for this species. Both chytrids appear to be saprophytic and incapable of parasitizing healthy normal cells. The former has been grown on cooked filaments of *Cladophora* sp. and *Oedogonium* sp. and to a limited extent on synthetic nutrient media in the laboratory, while the latter grows readily on dead internodes of *Nitella flexilis* and others members of the Characeae.

### *Chytridium aggregatum* sp. nov.

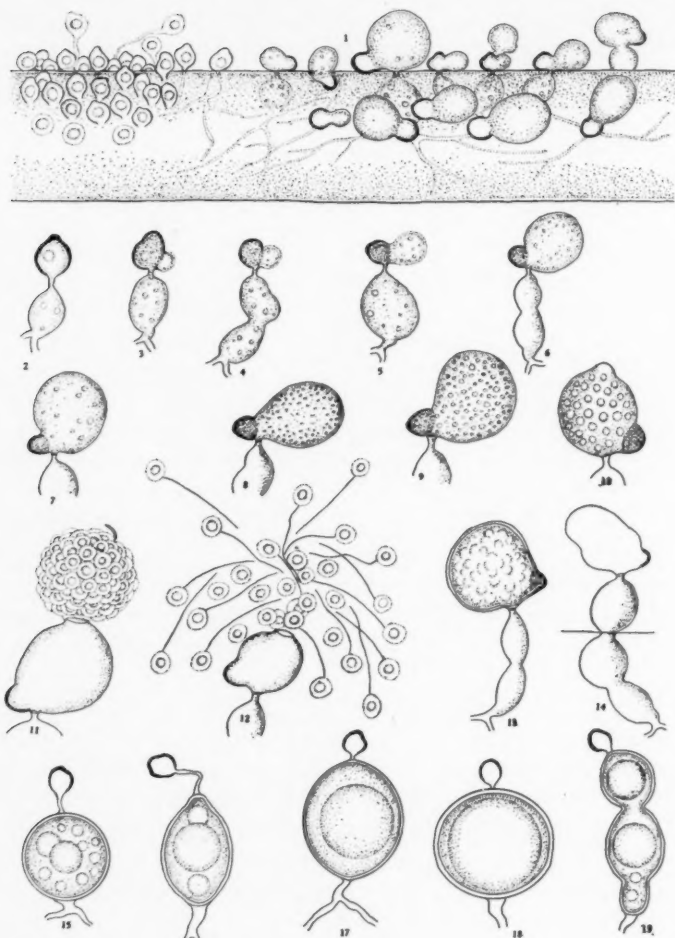
Thalli numerous and gregarious, partly intra- and extramatrical, eucarpic. Zoösporangia extramatrical, formed as a lateral or slightly basal outgrowth from the encysted zoöspore case and delimited from the apophysis by a cross wall at maturity; hyaline, smooth, oval, egg-shaped, subspherical,  $4 \times 6 \mu$ – $10 \times 18 \mu$ , with a conspicuous amber or brown protuberance, the zoöspore case, near the base, and an apical or slightly sub-apical exit papilla; operculum spherical,  $4$ – $8 \mu$ , or slightly oval. Zoöspores hyaline, con-

spicuously uniguttulate, spherical,  $3-4.5\ \mu$ , posteriorly uniciliate; emerging in a globular mass and lying quiescent near the exit papilla for a few moments before moving apart; motility confined to a few jerky motions and amoeboid movements; settling down on the host cell and germinating in a mass in the vicinity of the zoösporangium. Apophysis intramatrical, spherical,  $5-10\ \mu$ , oval, broadly spindle-shaped, elongated and occasionally constricted. Rhizoidal system well developed and branched, extending often to a distance of  $110\ \mu$ ; main axis  $3-4\ \mu$  in diameter. Resting spores intramatrical, hyaline, spherical,  $5-14\ \mu$ , oval, slightly citriform,  $6 \times 9\ \mu-12 \times 14\ \mu$ , somewhat depressed, and occasionally flattened or constricted, usually with a single large refractive globule and a  $2.5-3\ \mu$  thick, smooth wall; germination unknown.

Thallis magnis atque congregatis, per partes intramatricalibus atque extramatricalibus, eucarpicis; zoosporangiis extramatricalibus, vagina zoosporae, a latere vel basi excretis et apophyside pariete maturitate disjunctis; hyalinis, levibus, ovatis, subglobosis,  $4 \times 6\ \mu-10 \times 18\ \mu$ , conspicuo electro-fusco tubere, vagina zoosporae, basi et papilla apici vel subapici praeditis; operculo globoso,  $4-8\ \mu$ ; vel ovato. Zoosporis hyalinis, manifeste uniguttulatis, globosis,  $3-4.5\ \mu$ , cilio posteriore praeditis; cumulo globoso emergentibus et aliquamdiu vicinis papillae quietis et semotis; moto paucis impetibus atque serpentibus motibus coercito; cellula hospitis statutis und cumulo vicinis zoosporangio germinatis. Apophyside intramatricali, globoso,  $5-8\ \mu$ , ovato, late fusiformi, longius facta et aliquando stricto. Systema rhizoidorum ramosa, usque ad  $110\ \mu$  saepe extensa; principe axi  $3-4\ \mu$  dia. Sporis perdurantibus intramatricalibus, hyalinis, globosis,  $5-14\ \mu$ , ovatis, citriformis,  $6 \times 9\ \mu-12 \times 14\ \mu$ , aliquantum depressis, und aliquando planis vel strictis, uno magno refracto globulo atque levi crasso pariete  $2.5-3\ \mu$  ferme praeditis; germinatione incomperta.

Saprophytic on *Spirogyra crassa*, *Oedogonium* sp., and *Cladophora* sp. in New Jersey and New York, U. S. A.

The gregarious association of the thalli and their development and structure are shown in figures 1-19. The zoöspores settle down on the host cell after undergoing a few jerky movements and usually germinate in groups (FIG. 1), and in their midst may sometimes be seen the remnants of the old sporangium from which they originated. Occasionally a number may germinate in the water at a short distance away and form long germ tubes which grow toward the host. Some of these thalli may penetrate the host and attain mature development with a portion of the apophy-

FIGS. 1-19. *Chytridium aggregatum*.

sis on the outside. As germination and growth proceeds, a fair number of the young thalli resting on the host wall may be crowded out and die in the competition for food and space, so that the number of mature thalli in a group is usually much smaller than the number of swarmspores which germinated.

However, as many as 162 sporangia in isolated groups have been found on a single *Spirogyra* cell. The zoöspore case, in the meantime, persists on the surface of the host, and as growth of the intramatrical portion of the thallus continues its wall begins to thicken and turns yellow and brown. This thickening is usually most pronounced at the apex and decreases toward the base, so that the zoöspore case frequently appears strawberry-shaped, with the refractive globule persisting within for some time (FIG. 2).

The following developmental stages are very similar to those of *C. Schenkii* and *C. gibbosum* described by Scherffel (6, 8), and a detailed account here would be superfluous. Furthermore, the direction of growth and development is at first endogenous, and it is not until after the intramatrical rhizoidal system and apophysis are well established that the incipient zoösporangium makes its appearance, as I have shown for *C. lagenaria*. The young sporangium buds out as a small hyaline blister or vesicle at the side and usually near the base of the brown extramatrical zoöspore case (FIG. 3), and the direction of growth and movement of accumulated protoplasm become reversed and exogenous. With the continued upward movement of accumulated material from the apophysis and rhizoidal system the incipient zoösporangium increases in size, and as this goes on the zoöspore case is gradually displaced to a somewhat basal and lateral position, as is shown in figures 3-11. As a result, the mature zoösporangia are often very similar in appearance to those of *C. Schenkii* and *C. gibbosum*, with the exception that the adherent portion of the zoöspore case is yellow to brown in color.

The mature sporangium opens by a spherical or slightly oval operculum, and as the zoöspores ooze out, the latter is usually carried up on top of the mass (FIG. 11). The period of emergence of the zoöspores varies from 40-90 seconds, and is dependent to a large degree on the size of the sporangia and the number of swarmspores. Occasionally a few spores may be left behind and emerge later. The emerged zoöspores lie quiescent in a globular mass for a short while and then gradually begin to move apart, and as this goes on the cilium becomes visible. After they have separated thus the cilium may undergo a few jerky movements, but they do not become actively motile and swim away.

These jerky movements fail to carry the zoöspore away to any significant distance, and they eventually begin to settle down on the host cell in the vicinity of the sporangium, as is shown in figure 12. When this behavior was first observed, it was believed to be due to a pathological condition of the chytrid or perhaps some unfavorable environment, but after watching it occur repeatedly over a long period of time, I am of the opinion that it may be an inherent specific character of the fungus itself. At any rate, this feeble motility doubtless accounts for the gregarious habit of *C. aggregatum*.

In addition to the sporangia described above, a few hyaline thick-walled dormant ones have been found, as is shown in figure 13. They appear to be formed in the same manner as the thin-walled evanescent sporangia and are subtended basally and laterally by an unexpanded portion of the brown zoöspore case. Sporangia of this type have also been observed by Sparrow (10) and myself (2, 3) in *C. lagenaria* and *Endochytrium operculatum*. They apparently give rise to zoöspore like the other sporangia, but I have not so far observed their "germination."

Occasionally zoöspores which have germinated in the water at some distance from the host gain a foothold and develop into mature thalli. In such cases the mature sporangium may stand off at some distance from the host wall and be subtended by an extra-matrical apophysis. Figure 14 shows a thallus with an apophysis consisting of three oval parts in tandem, one of which is extra-matrical and supports the sporangium. Such thalli are strikingly similar in this respect to those of *Phlyctochytrium Zygnetis* described by Rosen (5).

The variations in size and shape of the resting spores are shown in figures 15-19. These spores are intramatrical and develop in the same manner as I have described for *C. lagenaria*. As the apophysis reaches mature size it becomes filled with accumulated protoplasm, particularly refractive substance, and develops a comparatively thick hyaline wall. Germination has not so far been observed, but it apparently occurs in the same manner as in *C. Olla* and *C. lagenaria*.

*Chytridium aggregatum* appears to be closely related to *C. Schenkii* and the form which Scherffel (6) calls *C. gibbosum*, be-

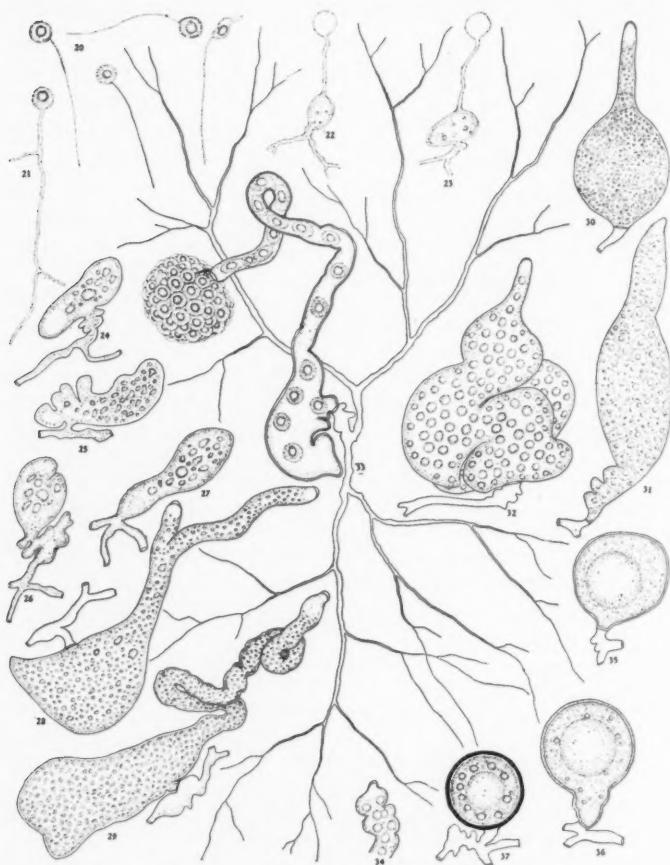
cause of the shape of the zoösporangium and the persistence of the zoöspore case near its base. In the latter two species, however, the swarmspore case, while appearing slightly dark and opaque, does not become brown in color. Furthermore, their zoöspores have been described as becoming actively motile, while those of *C. aggregatum* are only feebly so and germinate close together.

***Endochytrium digitatum* sp. nov.**

Thalli numerous, intramatrix, monocentric, eucarpic. Zoösporangia hyaline and smooth except for one to several blunt digitations at or near the base; formed as an enlargement on the germ tube and delimited from the rhizoidal system by a cross wall at maturity; elongate and obclavate  $11 \times 44 \mu$ – $18 \times 35 \mu$ , pyriform,  $15 \times 22 \mu$ – $71 \times 120 \mu$ , obpyriform, irregular, subspherical, somewhat triangular and lobed, with 1–4, usually one, single or branched, straight, curved, undulating, or coiled, tapering exit tubes,  $5$ – $18 \mu$  in diameter and  $10$ – $275 \mu$  in length, which may occasionally extend  $88 \mu$  beyond the surface of the host wall. Operculum spherical or slightly oval,  $3.3$ – $5.5 \mu$ . Zoöspores hyaline with a clear refractive globule  $1.6$ – $2.2 \mu$  in diameter, spherical,  $4.4$ – $5.5 \mu$ , posteriorly uniloculate; emerging fully formed and singly, and lying quiescent in a globular mass a short while before becoming actively motile, intermittently amoeboid. Rhizoidal system well developed and richly branched, extending sometimes for a distance of  $550 \mu$ , smooth or irregular in contour,  $2.7$ – $5 \mu$  in diameter and occasionally digitate at the base. Resting spores smooth, light to medium brown, oval, subspherical,  $16 \times 18 \mu$ – $10 \times 15 \mu$ , spherical,  $20 \mu$ , obpyriform, with a  $1.75$ – $2.5 \mu$  thick wall and a large refractive globule usually surrounded by several small ones; germination unknown.

Thallis magnis, intramatrix, monocentricis, eucarpicis. Zoösporangia hyalina, levibus, uno usque pluribus obtusis partitionibus apud vel prope basem exceptis; incrementis in tubulo formati et systema rhizoidorum pariete maturitate disjunctis; longius factis et obclavatis  $11 \times 44 \mu$ – $18 \times 35 \mu$ , pyriformis,  $15 \times 22 \mu$ – $71 \times 120 \mu$ , obpyriformibus, irregularibus, subglobosis, aliquantum triangularibus et rotundis, uno usque quattuor, solis vel ramosis, directis, flexis, undulatis vel tortuosis, cereis tubulis dimissionis,  $5$ – $18 \mu$  dia. atque  $10$ – $275 \mu$  long. praeditis,  $88 \mu$  trans superficiem parietis hospitis aliquando extensis. Operculo globoso vel ovato,  $3.3$ – $5.5 \mu$ . Zoösporis hyalinis, uno pellucido refracto globulo praeditis  $1.6$ – $2.2 \mu$  dia., globosis,  $4.4$ – $5.5 \mu$ , cilio

posteriore praeditis; adultis singillatim emergentibus et cumulo globulo aliquamdiu quietis et serpentibus motibus tempore celerime semotis. Systema rhizoidorum ramosa, usque ad  $550\ \mu$  saepe



FIGS. 20-37. *Endochytrium digitatum*.

extensa, levi vel irregulare,  $2.7-5\ \mu$  dia. et partitionibus basi praedita. Sporibus perdurantibus levibus, claris ad mediis fuscis, ovatis, subglobosis  $16 \times 18\ \mu-10 \times 15\ \mu$ , globosis,  $20\ \mu$ , obpyriformibus,  $1.75-3\ \mu$  pariete crasso atque magno refracto globulo pluribus parvis globulis circumdato praeditis; germinatione incompleta.



Saprophytic in dead internodes of *Chara coronata*, *Nitella flexilis*, and other algae in New Jersey and New York, U. S. A.

The life history and development of this species is fundamentally similar to *E. operculatum*, as is shown in figures 20-37. The zoöspores are similar in appearance and behavior, but possess a somewhat larger refractive globule than the type species (FIG. 20). Their method of germination and the early developmental stages of the rhizoidal system and sporangium (FIG. 21-23) appear to be the same, and no outstanding differences have yet been observed. Shortly following the stage shown in figure 23 the zoöspore case and germ tube gradually disintegrate and disappear, and so far no appendiculate sporangia such as occur in *E. operculatum* have been seen. Figure 24 shows an early stage in which the digitations have appeared on the main axis of the rhizoidal system, while in figure 25 they occur at the end and on the upper surface of the incipient sporangium. In figure 26 they are present on both, but the portion of the rhizoidal system immediately underneath the sporangium has become so expanded as to appear like an irregular, lobed apophysis. Somewhat similar structures are also present in the thalli shown in figures 29, 32, and 33, but in figures 27, 28, and 30 the digitations are lacking entirely.

The young sporangia (FIG. 24-27) are usually vacuolated and contain a number of large, globular and irregular refractive bodies which give the protoplasm a coarse refringent appearance, but as development proceeds, these bodies appear to break up into smaller and smaller fragments (FIG. 28). Eventually the protoplasm attains the finely granular, greyish refractive appearance shown in figure 29. Following this stage the granules may become grouped into more or less polygonal patterns (FIG. 30) which gives the impression that the protoplasm has undergone cleavage into polyhedral segments. Whether or not cleavage occurs at this stage or later is difficult to determine in living material, but in subsequent stages (FIG. 31) the lines of demarkation become less distinct. Furthermore, the refringent granules begin to coalesce into larger ones until a more or less definite number of equal sized, large and highly refractive globules are formed, as is shown in figure 32. These late maturation stages are strikingly similar to those which I have described in *Nephrochytrium appendiculatum* (4). The

condition shown in figure 33 may last from one to several hours, but eventually the operculum is pushed up and the zoöspores emerge. The operculum is usually persistent at the side of the orifice or may be found in its immediate vicinity. The initial behavior of the zoöspores is the same as in *E. operculatum*, emerging in a globular mass and lying quiescent for a short while before swimming away.

The wall of the sporangium and rhizoids do not give a marked cellulose reaction with chloro-iodide of zinc, but the exit tubes stain deeply lavender and violet at their extremities. The intensity of the reaction increases progressively from the base toward the tip in the same manner as Scherffel (7) has described for species of *Ectrogella*. The wall doubtless undergoes a marked change in composition with age and maturity, and as a result only the younger and more recently formed portions of the thallus show a marked cellulose reaction.

The development of the resting spores is much the same as in *E. operculatum*. The incipient spore usually contains a large number of comparatively small refractive globules which gradually coalesce to form a large central one (FIG. 35) as the wall begins to thicken. The latter is usually hyaline at first, but becomes yellow and finally light to medium brown with maturity, as is shown in figures 36 and 37. At the same time a few small refractive globules usually appear around the larger central one. The base of the spore may sometimes be irregular (FIG. 36) and show some indications of digitation. So far no sexuality has been observed in relation to their development.

With the addition of the present fungus, the genus *Endochytrium* includes four species, two of which are doubtful and possibly synonymous. Domjan's (1) *Entophlyctis pseudodistomum* should be transferred to this genus inasmuch as the sporangia are operculate. The persistence of the zoöspore case as well as the structure and development of the thallus are strikingly similar to *Endochytrium operculatum*, and since it is also saprophytic in decaying algal filaments and vegetable debris, it is not improbable that the two species may be identical. The validity of Sparrow's (9) *E. oöphilum* is contingent on its occurrence in eggs of rotifers, but since *E. operculatum* likewise inhabits the cysts of various

small animals it is possible that Sparrow's fungus may relate to this species also. As has been noted before, the main axis of the rhizoidal system immediately beneath the sporangium in *E. digitatum* may occasionally become somewhat irregular and inflated and have the appearance of an apophysis. This tendency may possibly foreshadow the evolution of the apophysis as it occurs in *Diplophlyctis*, and on this basis *E. digitatum* might perhaps be regarded as a transitional species in relation to this structure.

## SUMMARY

*Chytridium aggregatum* occurs as a saprophyte on dead and decaying filaments of *Spirogyra crassa*, *Cladophora* sp., and *Oedogonium* sp. in New Jersey and New York and has been cultured to a limited extent on synthetic nutrient media. It is characterized chiefly by the persistent brown zoöspore case, feeble motility of the swarmspores, and a pronounced gregarious habit of association. In the shape of the sporangia and the presence of the unexpanded portion of the zoöspore case as a protuberance near the base, this species is very similar and possibly closely related to *C. Schenkii* and *C. gibbosum*.

*Endochytrium digitatum* is a saprophyte in internodes of *Chara* and *Nitella* and may be readily grown on cooked filaments of various algae. It is distinguished from the other species of this genus by the presence usually of one to several blunt digitations near the base of the sporangium or on the main axis of the rhizoidal system and light to medium brown, smooth resting spores. In most of its other characters it is similar to *E. operculatum*. The main axis of the rhizoidal system at the base of the sporangium may often become slightly inflated and irregular and have the appearance of an apophysis.

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#### EXPLANATION OF FIGURES

*Chytridium aggregatum*. Fig. 1, a free hand sketch showing the gregarious habit of this chytrid; 2, showing the thickening and browning of the persistent zoöspore case; 3-6, successive stages in the development of the incipient zoösporangium as an outgrowth from the side of the zoöspore case; 7-10, stages in the maturation of the sporangium; 11, showing the emergence of the zoöspores in a globular mass; 12, showing the separation of the zoöspores and their settling down on the host in the vicinity of the sporangium; 13, a thick-walled dormant sporangium; 14, portion of a thallus with a peculiar lobed apophysis which is partly extramatrical; 15-19, variations in the size and shape of the resting spores.

*Endochytrium digitatum*. Fig. 20, showing the structure of the zoöspores; 21-23, stages in germination and the development of the zoösporangium as an enlargement of the germ tube; 24-26, young sporangia showing the origin of the digitations; 27-32, stages in the development and maturation of the zoösporangium; 33, a complete, small thallus showing the rhizoidal system and the emission of the zoöspores; 34, the inflated and irregular tip of an exit tube; 35-37, late stages in the development of the resting spores.

## TWO NEW SPECIES OF OMPHALIA WHICH CAUSE DECLINE DISEASE IN DATE PALMS<sup>1</sup>

DONALD E. BLISS

(WITH 10 FIGURES)

The species of *Omphalia* which constitute the subject of this paper are now considered (2) to be the cause of decline disease in the date palm, *Phoenix dactylifera* L. This malady was first detected in 1921 near Indio, California. Since that time the disease has become well distributed (4) throughout the date-growing region of the Indio district, but it is unknown beyond the boundaries of Riverside County. All underground parts of the palm (3) may be attacked by these fungi, but the principal injury results from the destruction of roots. Secondary symptoms (1) appear subsequently and include the premature death of leaves, retardation in the rate of terminal growth, and reduction in the size of leaves and fruitstalks. The fruit from severely affected palms is nearly worthless.

Among the microorganisms associated with diseased palms were certain white, sterile fungi with clamp connections at the septa. The first of these cultures was isolated in 1931 by L. J. Klotz, who obtained slight evidences of infection from the inoculation of seedling date palms. Little significance was placed on these results at that time. Later work by the author (2) revealed the pathogenic nature of Klotz's culture, together with that of numerous isolates of similar character from other diseased palms.

Since no fruiting bodies of these basidiomycetous fungi were found about date palms affected with decline disease, effort was directed toward inducing sporulation artificially. A group of imperfect toadstools developed in the greenhouse on a wooden pot label (FIG. 3, C). A moldy leaf base from a diseased palm had been buried in soil near the base of this marker. Mycelium which

<sup>1</sup> Paper No. 380, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

was obtained from the cap of one of these sporophores resembled closely in appearance and pathogenicity the culture which had been obtained from an active lesion in this diseased palm.

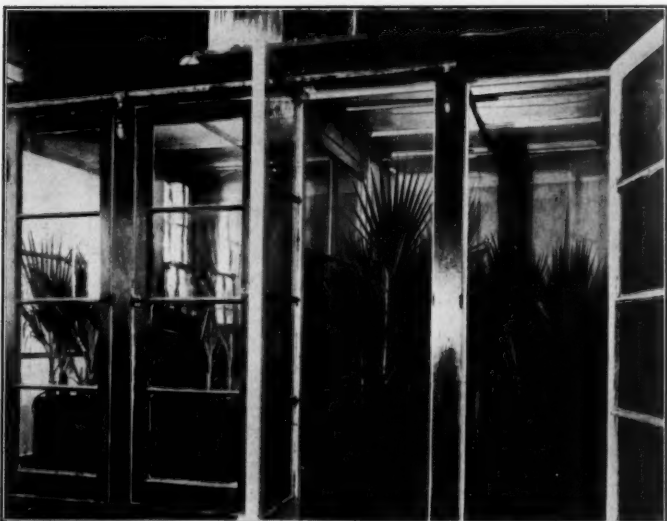


FIG. 1. Moisture chambers in headhouse of Plant Pathology greenhouse, University of California Citrus Experiment Station, with potted seedlings of *Washingtonia filifera* from which sporophores of *Omphalia* spp. were obtained.

The technique of Long and Harsh (6) was employed in an effort to induce sporulation in pure culture. Six isolates of different origin were grown, respectively, on malt, corn meal, prune, and carrot agar slants in 20-cm. test tubes. The reaction of these media ranged from pH 5.52 to 5.88 after sterilization. Although four series of these cultures were grown for a period of eight months at room temperature both in the dark and exposed to sunlight in three different positions, no fruiting bodies developed.

The first perfect sporophore (FIG. 7, E) appeared at the base of a potted seedling date palm in the greenhouse and was brought to full development in a moist chamber. A culture which was obtained from hymenial tissue of this toadstool was considered identical in appearance and pathogenicity to the culture used in the original inoculation.

Later it was found that sporulation could be induced more readily on seedlings of *Washingtonia filifera* Wendl. than on young date palms. Humidity and temperature also proved to be important factors, and there seemed to be some seasonal influence on fruiting. Although collections of toadstools were made during the period from January to October, the most abundant sporulation was obtained in the months of May to August, inclusive.

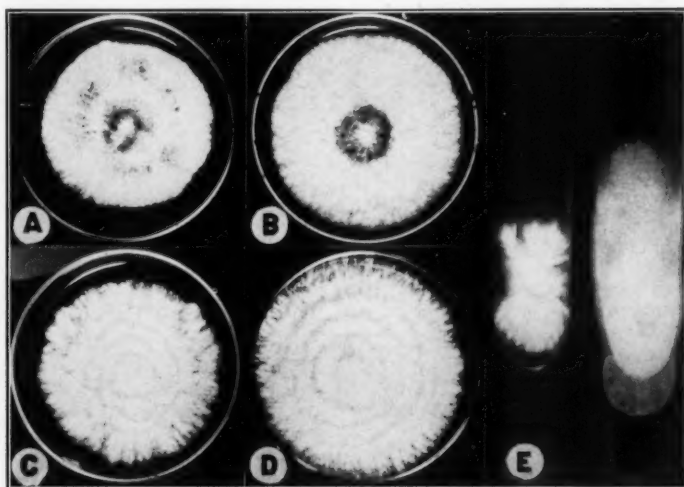


FIG. 2. Mycelial cultures of *Omphalia* spp. A and B, *O. tralucida* grown for 190 hours at 25° C. on a special agar medium containing date palm decoction ( $\times 0.35$ ); C and D, *O. pigmentata* grown under similar conditions ( $\times 0.35$ ); E, two types of mycelial growth of *O. tralucida* ( $\times 0.75$ ).

The present method used for inducing sporulation is as follows: Seedlings of *Washingtonia filifera*, planted in five-gallon containers in midsummer, are moved in November to the greenhouse. A pure culture of the fungus grown on 80 cc. of sterile Pillsbury's bran is mixed with the upper one-inch layer of soil in each container. The outer leaves of the palms usually begin to wilt and die within a month from the time of inoculation. Sporophore initials may appear at any time thereafter, arising from the bases of the dead outer leaves. Moist chambers (FIG. 1) are employed to obtain the fullest development of the toadstools. The tempera-

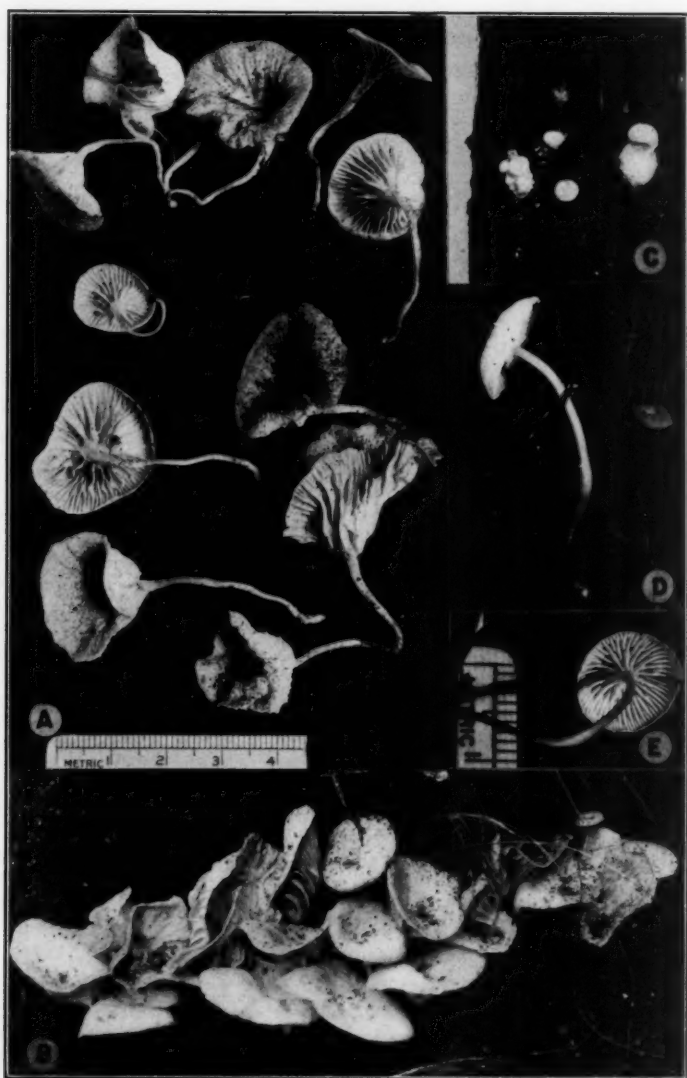


FIG. 3



ture is raised by means of a 200-watt electric lamp and reflector above each chamber and moisture is added by means of atomizers. The most favorable environment for the development of sporophores seems to be one in which the soil and air temperatures are maintained between 26° and 31° C. and the relative humidity of the air is held between 92 and 98 per cent. The fruiting bodies pictured in figure 3, *D* and *E*, and in figure 7, *A-D*, *F*, and *G*, were produced in this manner.

Because of the small size and fragile nature of the toadstools, it was found desirable to preserve some of them in a liquid such as the alcohol-formalin-acetic solution No. 2 of Rawlins (7). Spore prints were obtained by placing the caps of freshly collected toadstools on glass slides in a moist chamber. Spores were mounted in lactophenol, and the cover slips were sealed with lactophenol gum, as described by Davis (5). Some mounts were stained with a hot solution of cotton blue in lactophenol.

Freshly discharged sporidia or bits of gill tissue were planted on corn meal agar to secure cultures of the toadstool fungi. These cultures were compared with the ones which had been used in the original inoculations and, so far as could be determined, each one resembled its respective parent culture in cultural characters and in pathogenicity. Because toadstools were secured on palms which had been inoculated with sporidial cultures, it was thought that these strains of fungi had been taken through all stages of their respective life cycles.

Comparisons of the fruiting bodies which were obtained from the different strains of decline-disease fungi revealed the presence of two distinct species. Since the writer is not aware of any previous collections or descriptions of similar fungi, they are here diagnosed and described as new species.

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FIG. 3. *Omphalia pigmentata*: *A*, 12 representative toadstools from group shown in *B* ( $\times 0.81$ ); *B*, group of sporophores (type specimens) arising from the base of a young Saidu date palm in the open ( $\times 0.79$ ); *C*, young, imperfect toadstools which developed from a wooden pot label ( $\times 2.56$ ); *D*, two fruiting bodies on a seedling of *Washingtonia filifera* in the greenhouse ( $\times 1.54$ ); *E*, a different view of larger toadstool in *D* showing stipe and gills ( $\times 1.46$ ).

## OMPHALIA PIGMENTATA

This species is typified by abundant, white, silky, rather coarse mycelium which resembles glass wool (or spun glass) when confined in a test tube. A characteristic pigment, ranging in color from light orange-yellow to cadmium orange (8), forms when the mycelium is grown at 20° to 30° C. in a 2 per cent agar slant culture containing potato starch and dextrose. This pigment forms in the zone of contact between the mycelium and the substrate, and it is confined mostly to the margin of the slanted surface. The reverse side of the culture is not darkened. Figure 2, C and D, shows the floccose and ringed appearance of two colonies which were incubated 190 hours at 25° C. and grown on a special agar medium containing date palm decoction. Figure 2, A and B, illustrates colonies of *Omphalia tralucida*, the other species mentioned in this paper, which grew under similar conditions.

On August 28, 1935, a group of 65 toadstools (FIG. 3, B) was found arising from the base of a young Saidu date palm at the Citrus Experiment Station. Certain representative specimens from this group are illustrated (FIG. 3, A). Discovered four days after a torrential rainstorm which was followed by a period of hot, humid weather, this collection constitutes the only known instance in which *Omphalia pigmentata* has sporulated under natural conditions in the open. Figure 3, D and E, shows two well-formed toadstools which developed from a diseased seedling of *Washingtonia filifera* in the greenhouse. Under artificial conditions this species has not fruited as readily as *O. tralucida*. The collection of August 28, 1935, has been selected as the type for the following description:

***Omphalia pigmentata* sp. nov.**

*Pileus* 5 to 33 mm. broad, pale orange-yellow (8) approaching white with age, the pigment more concentrated at the center than at the margin, convex at first, applanate to infundibuliform when fully expanded (FIG. 3, A and B), umbilicate, membranaceous, tenacious, subdiaphanous, glabrous, striate-sulcate; margin inflexed, occasionally straight or reflexed, entire to undulate;

*Stipe* 5 to 35 mm. long, 0.5 to 2 mm. in diameter, compressed and enlarged near the apex, subalbulous, flexuous, abrupt, central

or somewhat eccentric, cartilaginous, glabrous, stuffed then fistulose (FIG. 4, *A*), caespitose or solitary;

*Lamellae* short-decurrent, thin, distant, simple, sometimes branched, unequal (FIG. 4, *A* and *B*), very pale orange-yellow to white;

*Basidia* (FIG. 5, *A*) 19 to 25 by 5 to 8  $\mu$ , hyaline;

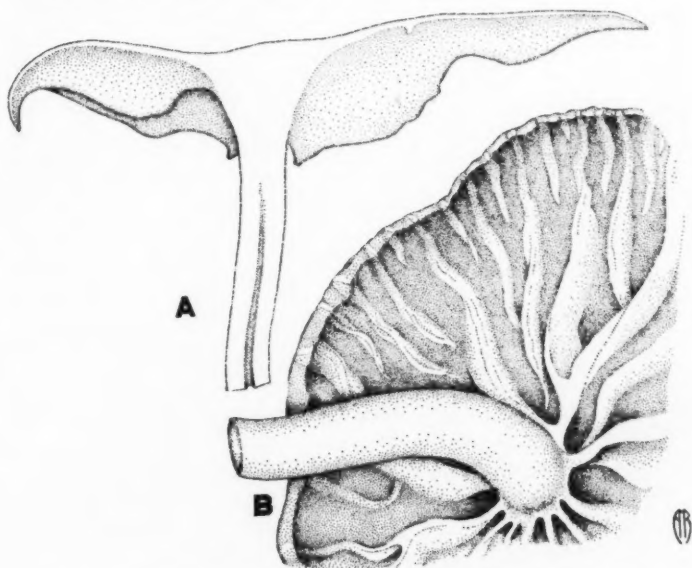


FIG. 4. *Omphalia pigmentata*: *A*, a median longitudinal section through a sporophore ( $\times 6.4$ ); *B*, part of a toadstool showing stipe and gill-bearing surface of pileus ( $\times 9$ ).

*Sporidia* 6 to 9 by 4 to 6.5  $\mu$  (FIG. 10), hyaline, white in mass, oval, papillate, even, germinating by means of a tube (FIG. 5, *B* and *C*);

*Hyphae* 1.1 to 6.5  $\mu$  in diameter, hyaline, branched, septate, with clamp connections at the septa (FIG. 5, *D*).

Pileo 5–33 mm. lato, ochroleuco ad album, convexo primo, deinde plano ad infundibuliformem, umbilicato, membranaceo, tenaci, subdiaphano, glabro, striato-sulcato; margine inflexo, nonnumquam recto aut reflexo, integro ad undulatum; stipite 5–35 mm. longo, 0.5–2 mm. lato, compresso et dilatato ad apicem, subalbo, flexuoso, abrupto, centrali aut eccentro, cartilagineo, glabro, solido deinde fistuloso, caespitoso aut solitario; lamellis breviter decurrentibus, tenuibus, distantibus, simplicibus, interdum ramosis, inaequalibus; basidiis 19–25  $\times$  5–8  $\mu$ ; sporis 6–9  $\times$  4–6.5  $\mu$ , hyalinis, ovatis, papillatis.

Collected on leaf bases of *Phoenix dactylifera* L. (type) and *Washingtonia filifera* Wendl. at Riverside, California.

Distribution: Riverside County, California.

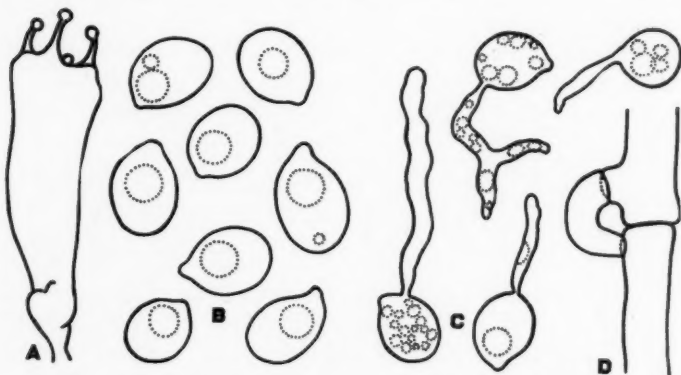


FIG. 5. *Omphalia pigmentata*: A, basidium with 4 young sporidia; B, discharged sporidia; C, germinating sporidia; D, clamp connection in a hypha. All drawings  $\times 1756$ .

*Type specimens* deposited with the Mycological Collections, Bureau of Plant Industry, Washington, D. C.; co-types sent to the Farlow Herbarium, Harvard University, Cambridge, Massachusetts, and the University of California Herbarium, Berkeley, California. A mycelial culture was placed at the Centraal Bureau voor Schimmelcultures, Baarn, Holland.

#### OMPHALIA TRALUCIDA

The mycelial growth of this species is white and comparatively fine in texture. When grown on slants of 2 per cent potato dextrose agar (FIG. 2, E), the mycelium may assume a loose, cottony appearance or else the hyphae may form small mats or fans over the substrate. The hyphal tips may bend backward against the inner surface of the test tube in an agar slant culture but they do not fill the tube so completely as do the aerial hyphae of *Omphalia pigmentata*. The reverse side of the culture may develop a brown to black discoloration but no yellow to orange pigment is formed.

Figure 6 shows rhizomorphs on segments of date palm roots after artificial inoculation. Under natural conditions rhizomorphs are much less conspicuous.

Although no sporulation has been observed in the open, more than 350 toadstools have been collected over a period of three years from experimental plants in the greenhouse and moisture chambers. While the sporophores are found usually alone or in groups of two or three, a relatively large number of fruiting bodies



FIG. 6. Rhizomorphs of *Omphalia tralucida* on segments of a living root of seedling date palm after artificial inoculation ( $\times 2.5$ ).

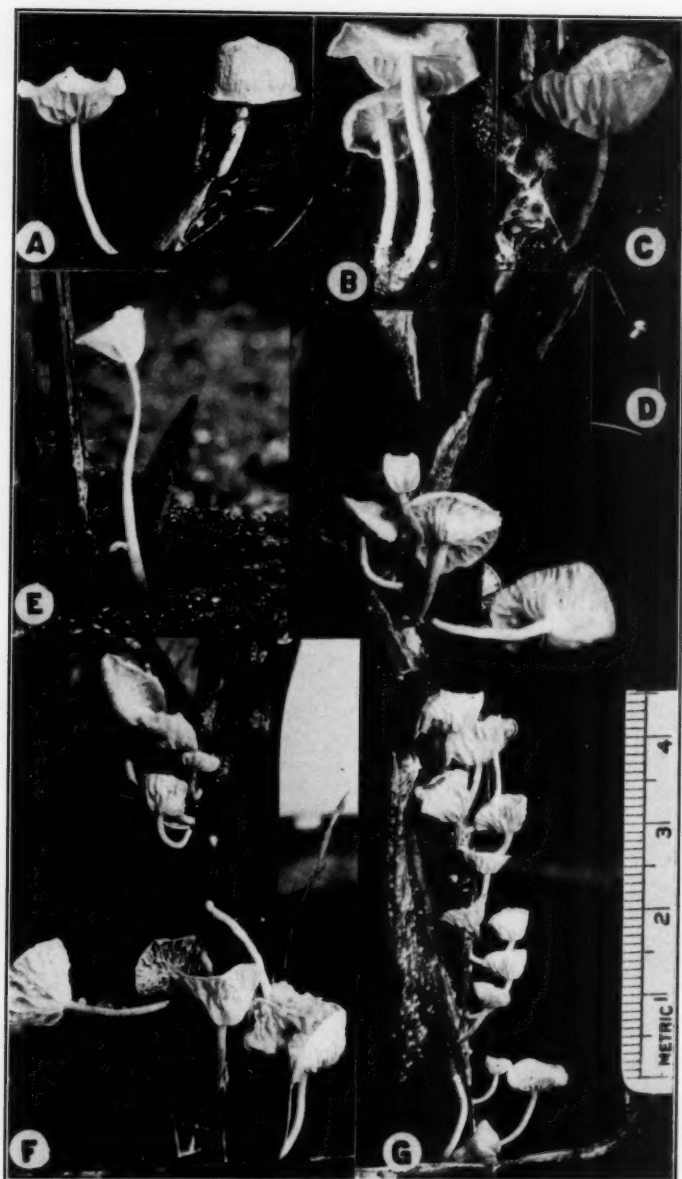


FIG. 7

expanded at one time on two seedlings of *Washingtonia filifera* (FIG. 7, *F* and *G*). These sporophores, together with those pictured in figure 7, *A*, *C*, and *D*, were obtained from one isolate, and they have been selected as the type material for the following description:

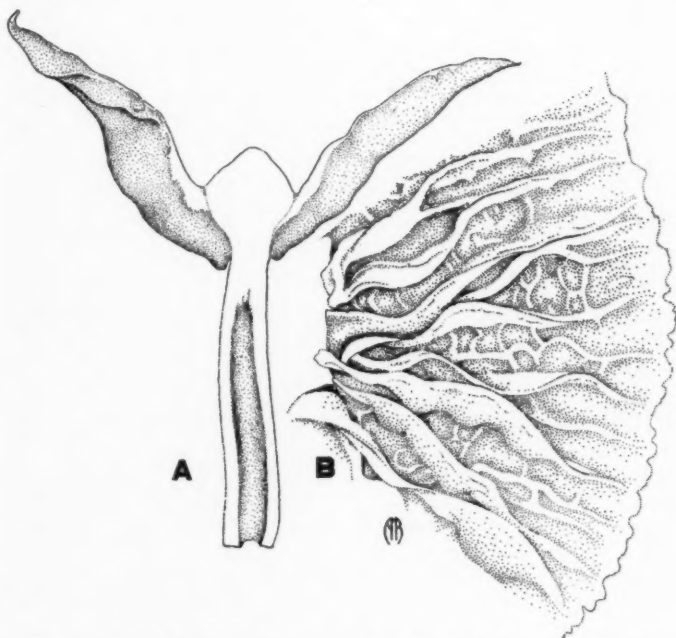


FIG. 8. *Omphalia tralucida*: *A*, a median, longitudinal section through a toadstool ( $\times 6.4$ ); *B*, a sector of the pileus showing the branched and inter-venous character of the lamellae ( $\times 9$ ).

***Omphalia tralucida* sp. nov.**

*Pileus* 3 to 18 mm. broad, white then cartridge buff (8), convex to infundibuliform, umbilicate, umbonate (FIG. 8, *A*), translucent, membranaceous, fragile, becoming flaccid; surface finely pubescent, striate-sulcate; margin straight or reflexed, sometimes inflexed, entire or subundulate;

FIG. 7. *Omphalia tralucida*: *A*, *C*, *D*, *F*, and *G*, sporophores (type material) arising from seedlings of *Washingtonia filifera* in the greenhouse ( $\times 1.3$ ); *B*, toadstools which developed on a seedling date palm ( $\times 2.95$ ); *E*, the first perfect fruiting body to be obtained ( $\times 2.6$ ).

*Stipe* 4 to 23 mm. long, 0.3 to 1.7 mm. in diam., cartilaginous, slender, curved, subequal stuffed then hollow (FIG. 8, *A*), white to cartridge buff, finely pubescent, abrupt, central, solitary;

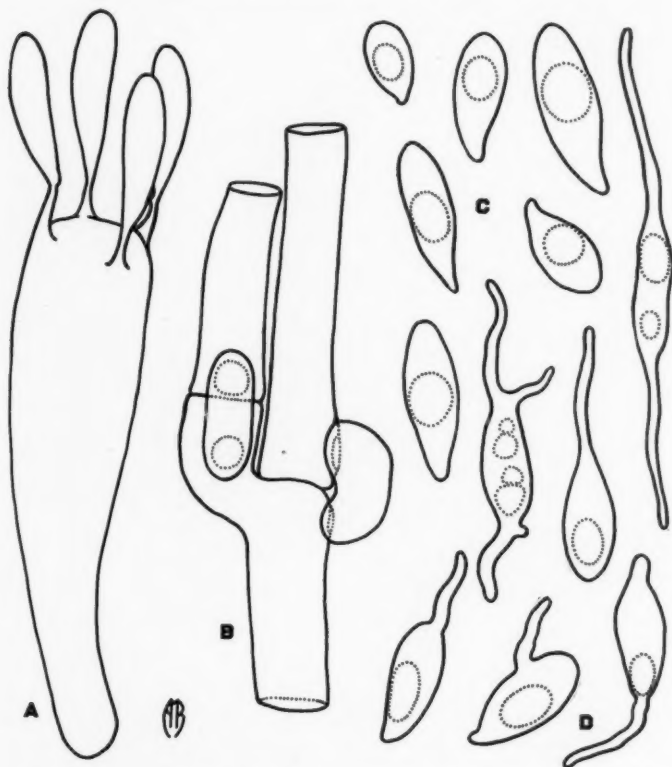


FIG. 9. *Omphalia tralucida*: *A*, basidium with four spordia; *B*, a branched hypha with two clamp connections at the septa; *C*, six discharged spordia; *D*, six germinating spordia. All drawings  $\times 1756$ .

*Lamellae* short-decurrent, sometimes attached only slightly, thick when young becoming thinner with age, distant, branched, intervenous (FIG. 8, *B*), unequal, white;

*Basidia* (FIG. 9, *A*) 32 to 46 by 6 to 12  $\mu$ , hyaline;

*Sporidia* 11 to 16 by 3 to 6  $\mu$  (FIG. 10), hyaline, white in mass, fusiform-ellipsoidal, papillate, germinating by means of a tube (FIG. 9, *C* and *D*);



*Hyphae* 0.9 to 6.7  $\mu$  in diameter, hyaline, branched, septate, with clamp connections (FIG. 9, B) at the septa.

Pileo 3-18 mm. lato, albo deinde stramineo, convexo ad infundibuliformem, umbilicato, umbonato, pellucido, membranaceo, fragili, postremo flaccido; superficie tenuiter pubescenti, striato-sulcato; margine recto aut reflexo, nonnumquam inflexo, integro aut subundulato; stipite 4-23 mm. longo, 0.3-1.7 mm. lato, cartilagineo, gracili, curvato, subaequali, solido deinde fistuloso, albo ad stramineum, tenuiter pubescenti, abrupto, centrali, solitario; lamellis breviter decurrentibus, interdum leviter coniunctis crassis denique tenuibus, distantibus, ramosis, intervenosis, inaequalibus, albis; basidiis 32-46  $\times$  6-12  $\mu$ ; sporis 11-6  $\times$  3-6  $\mu$ , hyalinis, fusiformis-ellipsoidis, papillatis.

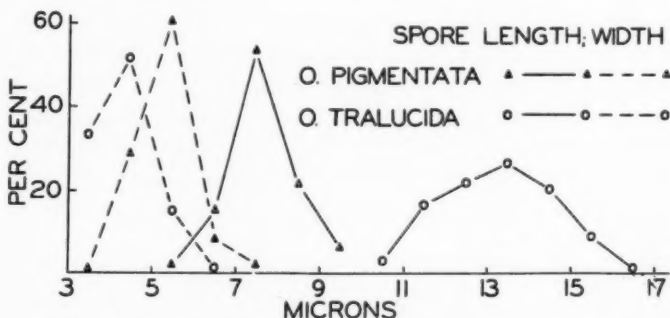


FIG. 10. Percentage distribution of different spore sizes. The curves for *Omphalia pigmentata* represent measurements of 250 sporidia while those for *O. tralucida* represent measurements of 400 sporidia.

Collected on leaf bases of *Washingtonia filifera* Wendl. (type), *Phoenix dactylifera* L., and *P. canariensis* Chaub. at Riverside, California.

*Distribution:* Riverside County, California.

Type specimens deposited with the Mycological Collections, Bureau of Plant Industry, Washington, D. C.; co-types sent to the Farlow Herbarium, Harvard University, Cambridge, Massachusetts, and the University of California Herbarium, Berkeley, California. A mycelial culture placed at the Centraal Bureau voor Schimmelcultures, Baarn, Holland.

#### SUMMARY

Cultures of two basidiomycetous fungi were isolated from the roots of date palms which were affected with decline disease. These fungi, which are considered to be the cause of the malady, do not fruit commonly in the open. A method is described by

which sporulation was obtained on inoculated seedlings of *Washingtonia filifera* in the greenhouse. Soil and air temperatures between 26° and 31° C. and a relative humidity of the air between 92 and 98 per cent were favorable environmental conditions for the development of sporophores.

These decline-disease fungi are diagnosed and described as new species. The principal differences between the two are as follows: The mycelium of *Omphalia pigmentata* resembles glass wool and produces a light orange-yellow to cadmium orange pigment when grown at 20° to 30° C. on slants of 2 per cent potato dextrose agar. The sporophores are relatively large and tough, while the sporidia are oval-shaped and measure 6 to 9 by 4 to 6.5  $\mu$ . Mycelium of *O. tralucida* may be distinguished from that of the other by a finer texture and the absence of the yellow to orange pigment. The reverse side of the culture may develop a brown to black discoloration. The toadstools are comparatively small and fragile, while the sporidia measure 11 to 16 by 3 to 6  $\mu$  and are fusiform-ellipsoidal in shape.

The writer wishes to express his appreciation to Olive H. Frost and E. L. Rea for suggestions in preparing the Latin descriptions.

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## NOTES ON SOME BASIDIOMYCETES FROM THE ORIENT

C. J. HUMPHREY

(WITH 3 FIGURES)

### THE GENUS *ELMERINA*

This genus was erected by Bresadola as *Elmeria*<sup>1</sup> in 1912 but in a later publication during the same year the name was changed to *Elmerina*.<sup>2</sup> Following is the diagnosis:

Fungi ex integro membranaceo-coriacei, pileati, hymenio lamellato, poroso-lamellato, vel daedaleoideo, dense setuloso, setulis pluricellularibus, ex hyphis conglutinatis. *Mycoboniae* analogum, prope *Daedaleam* in systemate locandum.

Cl. A. D. E. Elmer, de fungis philippinensibus optime merito, jure dicatum genus.

Obs. Genus hoc a genere *Tilotus* Kalchbr. prorsus diversum. In genere *Tilotus villositas* lamellarum e setulis simplicibus, unicellularibus, cystidioidis, apice granulosis, efformata, et meo sensu, vix setulae genuinae, sed basidia sterilia prout observantur in *Stereis* plurimis uti *Stereum concolor* Berk., *Stereum princeps* Jungh., *Stereum spectabile* Kl., etc.

The genus at present contains six species and one variety: *E. Berkeleyi* (Sacc. & Cub.) Petch, *E. cladophora* (Berk.) Bres., *E. flabelliformis* (Berk.), *E. foliacea* Pat., *E. setulosa* (P. Henn.) Bres., *E. setulosa* var. *Reyesii* Pat., and *E. vespacea* (Pers.) Bres. It was accepted by Patouillard but not by Lloyd. Petch<sup>3</sup> considered it doubtful whether it would be maintained.

It is a rather nondescript group since the various species partake of the superficial characters of *Hexagona*, *Lenzites*, *Trametes*, *Cyclomyces*, *Cladoderis*, and to a lesser extent of *Panus*. The one character the species have in common is the clothing of the hymenial surface with many pluricellular bristles composed of agglutinated hyphae, analogous to those in *Mycobonia*. Lloyd<sup>4</sup>

<sup>1</sup> Hedwigia 51 (1911): 318. Jan. 25, 1912.

<sup>2</sup> Ann. Myc. 10: 507. 1912.

<sup>3</sup> Ann. Roy. Bot. Gard. Perad. 9: 128. 1924.

<sup>4</sup> Myc. Writ. 7: 1153. July, 1922.

reports that he also found a typical *Lentinus* and a *Favolus* with such "setae." For the most part the species are so common that they may warrant segregation even if only on one common, but conspicuous, character; however, the final decision should be held open until more is known of the structure of tropical *Polyporaceae*.

ELMERINA BERKELEYI (Sacc. & Cub.) Petch.

*Panus coriaceus* Berk. & Br. Jour. Linn. Soc. (Bot.) 14: 45.  
Oct. 9, 1873.

*Hexagona flabelliformis* Berk. Jour. Linn. Soc. (Bot.) 16: 47.  
May 31, 1877.

*Hexagona cladophora* Berk. Jour. Linn. Soc. (Bot.) 16:  
47-48. May 31, 1877.

*Elmerina Berkeleyi* was described as *Panus coriaceus* Berk. & Br. (non *Panus coriaceus* Berk. from Australia). It was renamed in Saccardo's *Sylloge Fungorum*.<sup>5</sup> Lloyd's *Panus coriaceus* from the Philippines is the same as *Elmerina flabelliformis* (= *E. cladophora*) and was so considered by Bresadola. Lloyd<sup>6</sup> thought it was not the same, however, and stated that it matched a collection sent by Petch from Ceylon, which Petch had compared with the type of *Panus coriaceus* Berk. & Br. at Kew. On the assumption that Lloyd is right in referring Philippine material to that species then *Elmerina Berkeleyi* becomes the name to apply to the species common to both countries, since it has priority of publication by three years.

*Elmerina flabelliformis* was described in 1877 from Malanipa Island, Philippines, collected on January 30, 1875, Challenger Expedition No. 220, as *Hexagona flabelliformis* Berk. *Elmerina cladophora* was described on the same date from Malamon Island, Philippines, collected on February 4, 1875, Challenger Expedition No. 221, as *Hexagona cladophora* Berk. This gives priority of publication to the former.

The two islands are near the southern end of Zamboanga Peninsula and their proximity to each other strengthens the view that they represent but a single species.

The type of *Hexagona flabelliformis* in Herb. Kew readily and

<sup>5</sup> Vol. 5, p. 628.

<sup>6</sup> Myc. Writ. 4: letter 56: 6. 1915.

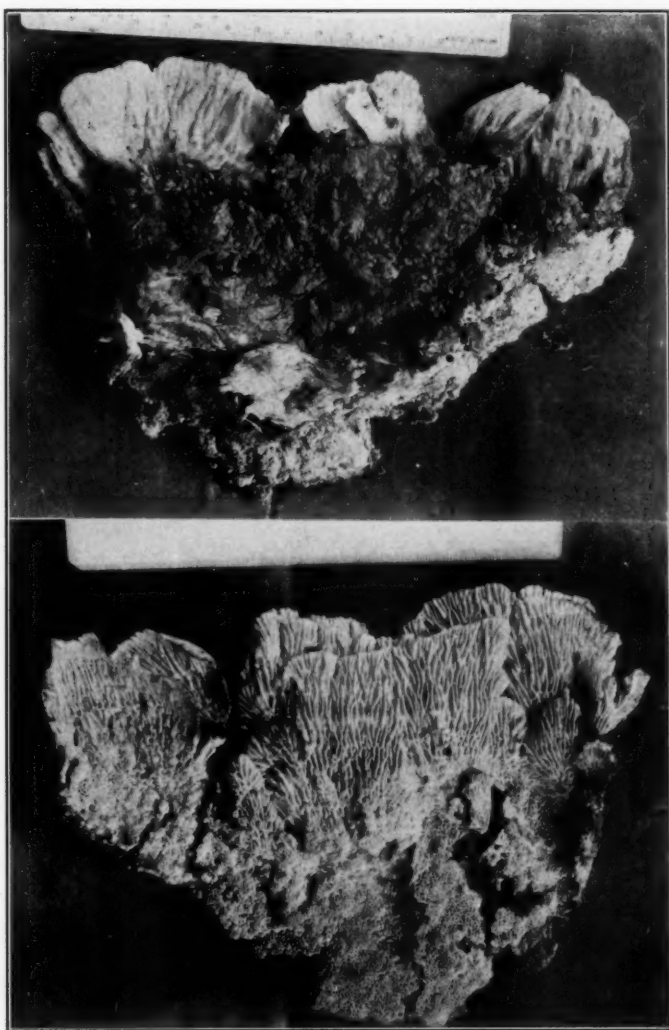


FIG. 1. *Elmerina Berkeleyi* (Sacc. & Cub.) Petch. Specimen freshly collected from a fallen broadleaf trunk, Tayabas Province, Luzon, P. I.  $\times 0.48$ , 2, lower surface of the specimen shown in figure 1.  $\times 0.48$ .

closely matches specimens from the Philippines in my own collection.

The type of *Hexagona cladophora* is somewhat larger, and sufficiently well preserved to permit interpretation. It apparently represents only part of a pileus, now strongly inrolled in drying. One portion has thick-walled, imperfectly formed, irregular pores over the entire lower (inner) surface, while a marginal fragment is sublamellate, with rather thin-walled dissepiments.

The species (FIGS. 1, 2) is normally thin pileate, with the pilei tough-fibrous, the upper surface somewhat puberulent and fibrous-striate, and the margin acute, entire to somewhat incised. It is white when fresh but the plants usually discolor to varying degrees of reddish brown, often becoming rather brittle and resinous in appearance. The inrolling of the dried specimens is usually very marked. The lower surface varies from poroid to lamellate, but in most lamellate specimens there are at least indications of large, shallow, irregular pores at the base.

At times the fungus forms large resupinate poroid sheets at the point of attachment of the pileus. I believe these are what Patouillard identified in the Baker herbarium as *Sistotrema autochthon* Berk. & Br.

#### ELMERINA FOLIACEA Pat.

The small specimen of the cotype<sup>7</sup> from Herb. Baker in my collection is rigid and hard, with the margin inrolled and lacerate. The upper surface is radiate-fibrous and covered with brownish pubescence behind. It was described as white when fresh but is now blackish-murinus in color. The under surface is sublamellate, with interrupted somewhat sinuous plates, and is almost concolorous with the upper surface. The context is hard and horny, almost black. Patouillard states the bristles are identical with those of *Elmerina cladophora*. The fungus considerably resembles *Polyporus durus* Jungh. in coloration and rigidity. It appears to be a good distinct species.

#### ELMERINA SETULOSA (P. Henn.) Bres.

This was described from East Africa as *Poria setulosa* P. Henn.<sup>8</sup> It is a resupinate coriaceous species readily separating

<sup>7</sup> Philip. Jour. Sci., C 10: 93. Mar. 1915.

<sup>8</sup> Engl. Bot. Jahrb. 28: 321. May 22, 1900.

from the substratum. It is buff in color, verging into light brownish, with rather regular, quadrangular to hexagonal pores averaging 3 to 4 in two millimeters. Some of the pores may appear linear owing to imperfect development, and consequent depression, of some of the cross walls.

*ELMERINA SETULOSA* (P. Henn.) Bres. var. *REYESII* Pat.

The type of this variety was originally identified by Patouillard from Baker's Philippine collection as *Elmerina setulosa*. He later described it as *Hexagona Reyesii*<sup>9</sup> but in his herbarium, now at Harvard University, he refers it as a variety of *Elmerina setulosa*. The specimen is rather young and not worthy of varietal distinction.

*ELMERINA VESPACEA* (Pers.) Bres.

Persoon's specimen of *Polyporus vespaceus*<sup>10</sup> came from the island of Rawak. It is a species widely distributed in the oriental tropics and is often rather large, thick, and light weight, white to buff when fresh. Some specimens are wholly poroid, with large, hexagonal, thin-walled pores; others are strictly lamellate, with broad and distant gills. The upper surface is often scurpy. The bristles of the hymenium are usually far less conspicuous than in the other species. It has many synonyms and is frequently filed in herbaria as *Hexagona albida* Berk. *Cyclomyces albida* Lloyd<sup>11</sup> is considered by its author merely as "a cyclomycoid form of *Hexagona albida*," hence should be placed in synonymy with *Elmerina vespacea*.

#### SOME GANODERMA SPECIES

While studying the *Ganoderma* group in the Philippines it was found necessary to make a few new combinations and reduce one species to synonymy.

<sup>9</sup> Leaf. Philip. Bot. 6, Art. 104: 2246. June 6, 1914.

<sup>10</sup> Freycinet, Botanique du Voy. autour du Monde . . . l'Uranie et la Physicienne pendant . . . 1817-1820, p. 170. 1826.

<sup>11</sup> Myc. Writ. 6: 1007. Sept. 1920.

**Ganoderma mirabile** (Lloyd) comb. nov.

*Fomes mirabilis* Lloyd Myc. Writ. 3: Letter 33: 3. May, 1911.

*Fomes fusco-pallens* Bres. Hedwigia 56: 294. Mar. 25, 1915.

The type of *Fomes mirabilis* was collected by C. B. Ussher in the Straits Settlements; the type of *Fomes fusco-pallens* is Merrill, 3693, from the Philippines. The spores are not globose, as both Bresadola and Lloyd state for their respective species, but ovate, brownish, and distinctly striate,  $5.7-6.8 \times 7.5-9.0 \mu$ , ten from the upper surface of Bureau of Science No. 50102 showing a mean of  $6.2 \times 8.4 \mu$ ; somewhat smaller when taken from a crushed mount of the pores. The context varies from rather hard to spongy.

Lloyd considered the two species the same but stated that the fungus was not a *Ganoderma*.

**Ganoderma subresinosum** (Murr.) comb. nov.

*Fomes subresinosus* Murr. Bull. Torrey Club 35: 410. Aug., 1908.

This species is a typical laccate *Ganoderma*, with pale context, but in his description Murrill gives the spores as "smooth, hyaline,  $3-4 \mu$ ." In eight of the specimens examined from the Philippines, Cambodia, and Ceylon a few spores were found in the crushed mounts of the pores. These were very large, ovate, thin-walled, olive buff to deep olive buff (Ridgway) under high power, with fine but distinct striations under oil immersion. They are so thin walled they frequently collapse in drying. A measurement of ten spores from each of the eight collections yielded a mean of  $10.6 \times 16.2 \mu$ , with extreme limits  $9.2-12.0 \times 13.4-19.7 \mu$ .

**Ganoderma hypoxanthum** (Bres.) comb. nov.

*Polyporus hypoxanthus* Bres. Ann. Myc. 10: 494. Oct. 31, 1912.

The type from Java in Herb. Bresadola at Stockholm, with a portion in Herb. Kew, is a small, slightly laccate, species with a rather hard buff context. Very few spores were found in crushed mounts of the pores, but these are ovate, as Bresadola states, near



olive buff (Ridgway) under high power, with moderately fine, distinct, striae,  $4.9-6.3 \times 6.3-7.8 \mu$ . The mean for five was  $5.7-7.0 \mu$ , larger than *Bresadola* indicates.

#### GANODERMA MINDOROI (Lloyd)

This was described by Lloyd as *Polyporus Mindoroi*.<sup>12</sup> The type collection in Herb. Kew is Copeland, 380, collected in the island of Mindanao, Philippines. The type packet is endorsed by Lloyd "*Polyporus mindanaoi* based on this collection," hence the use of the specific name *Mindoroi* was apparently an error. However, it is just as good a name, as the fungus is widely distributed in the Philippines. It belongs in a group of forms having long-ovate, rather finely striate spores, averaging around  $5 \times 11 \mu$ . Patouillard's references to *Ganoderma Mangiferae* Lév., which does not occur in the Philippines, are mainly this species as recorded in Philippine herbaria. Bresadola referred the forms principally to *Ganoderma cupreum* (Fr.) and *Ganoderma lingua* (Nees). I am not yet sure as to the correct name to apply but *Ganoderma Mindoroi* will eventually be reduced to synonymy.

#### GANODERMA DORSALE Lloyd

This species was described from Brazil as *Polyporus* (*Gan.*) *dorsalis*,<sup>13</sup> and specimens from the Philippines so referred by Lloyd are for the most part *Ganoderma amboinense* (Lam.). Since Lloyd placed this species in his section *Ganodermus*, which represents *Ganoderma*, no new combination is warranted.

#### EXIDIA LAGUNENSIS Graff<sup>14</sup>

The type collection (FIG. 3) is Bureau of Science No. 20972, collected from decaying wood on Mount Maquiling, Laguna Province, Luzon, Philippines, in 1912.

An examination of this shows it is not an *Exidia* but a member of the *Dacrymycetaceae*, namely, *Guepinia Spathularia* (Schw.). It is in good agreement, both macroscopically and structurally,

<sup>12</sup> Myc. Writ. 7: 1261. Jan. 1924.

<sup>13</sup> Myc. Writ. 5: 658. Apr. 1917.

<sup>14</sup> Philip. Jour. Sci., C 8: 299. Nov. 1913.

with that species as well as with *Guepinia ramosa* Curr. and *Guepinia fissa* Berk., both of which are considered forms of *Guepinia Spathularia*. Only a few spores were found in the type but these conform with the description and figures of Coker<sup>15</sup> for *G. Spathularia*, although somewhat smaller. The mean for five

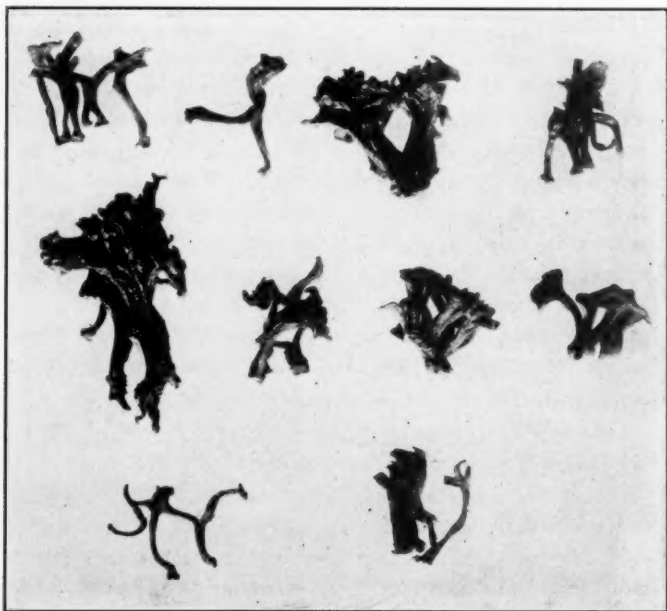


FIG. 3. Type collection of *Exidia lagumensis* Graff after the specimens were soaked out and separated. Approx.  $\times 2.0$ .

measurements was  $3.7 \times 7.9 \mu$ , while Coker gives them as  $3.8-4.5 \times 7.4-9.5 \mu$ . Some were two-celled. The basidia noted were rather young, nonseptate, with thick bifurcate tips (sterigmata), with a mean size of  $2.8 \times 26.3 \mu$ .

I am unable to reconcile Graff's description with the specimens preserved as the type. They were poorly dried into a distorted mass, which I soaked out, separated and photographed.

<sup>15</sup> Jour. Elisha Mitch. Sci. Soc. 35: 177, Pl. 64. June, 1920.

ACKNOWLEDGMENTS

Acknowledgments are due Mrs. Simeona Leus-Palo for her assistance in examining the specimens of *Ganoderma* and *Exidia*. The photograph of *Exidia* was taken by the photographic division of the Bureau of Science, Manila. A joint paper on the Philippine species of *Ganoderma* is planned and this will present illustrations and full descriptions of that group.

## NOTES ON THE MYCETOZOA—II

ROBERT HAGELSTEIN

I am recording here the rarely found, interesting, or unusual species, varieties, and phases of the Mycetozoa collected by myself and members of my staff, Joseph H. Rispaud, Leon J. Chabot, and John D. Thomas, during the season of 1937. Specimens of the same nature and received from friends and correspondents are also mentioned. When not otherwise stated, the collections described were made in 1937; and, unless another collector is named, the gatherings were found by Mr. Rispaud and myself in company.

Satisfactory weather conditions prevailed throughout the summer so that collections were abundant, and a number of species were discovered by us that had not been found heretofore in the different areas covered again. A curious general condition was noted. Certain species, among which were *Physarum cinereum* (Batsch) Pers., *Didymium squamulosum* (Alb. & Schw.) Fries, and the common species of *Arcyria*, were in no way as plentiful as in other years. On the other hand, *Diderma simplex* (Schroet.) Lister, *Physarum virescens* Ditmar, and *Badhamia lilacina* (Fries) Rost. were abundant everywhere, springing up in thousands of colonies as if by magic. For some years past, these forms have been seen occasionally only, and the sudden appearance in such great numbers is incomprehensible. On Long Island in a moist, suitable area it was not unusual when searching the ground carefully to find a fruiting of *D. simplex* in each square yard. A similar situation prevailed there about ten years ago.

It is gratifying to notice a considerable increase in the number of active students engaged in the collection and study of the Mycetozoa. During the past year I have been in contact, personally or through correspondence, among others, with Travis E. Brooks of Kansas, Lloyd G. Carr of Virginia, William D. Gray

of Indiana, Roy F. Cain of Ontario, Miss Charlotte B. Buckland of Florida, Frederick P. McIntosh of New York City, Fred Schunk of Pennsylvania, and Robert O'Connor of Staten Island. These students deserve particular mention because of their intensive collecting efforts, resulting in the uncovering of several rare or little known species, some of which are referred to in this paper. Let the good work continue.

*ARCYRIA INSIGNIS* Kalch. & Cooke. Numerous and extensive developments of var. *dispersa* Hagelstein (*Mycologia* 21: 298-299, 1929) were found by Mr. Rispaud on ground leaves near Amityville, Long Island, in August. Generally, the fruitings were on the inside of curled and twisted dry oak leaves under isolated scrub oak trees and exposed to the sun's rays. The open locality is similar to that at Jones Beach, Long Island, from where the variety was first reported. The variety differs from the typical form in that the sporangia are scattered, not clustered. N. Y. B. G. No. 1908.

*ARCYRIA OCCIDENTALIS* (Macbr.) Lister. Typical examples from the Eastern States seem to be rare. A fine collection made by Mr. Fred Schunk at Chinchilla, Pennsylvania, in October 1935, shows every sporangium with the persistent peridium dividing into the characteristic lobes. Several collections made personally in Pike and Wayne Counties, Pennsylvania, in October 1936, show the lobes fairly well developed. These fruitings are on dead poplar wood, sometimes beneath the distended bark. The sporangia are crowded, erect, stalked, and of an olivaceous-yellow or ochraceous color. The capillitium is yellowish.

There is an inclination among students to regard as phases of this species certain darker colored, more or less recumbent or irregular forms, basing determinations on a critical diagnosis of the capillitium and spores. Little dependence can be placed on those characters. Such forms are often imperfectly developed phases of *Arcyria stipata* (Schw.) Lister, particularly if there are indications of a calyculus, although there are intermediates which are practically indistinguishable. It is better to regard the peridium with its lobes as the important character. The habitat on poplar probably accounts for the scarcity of typical developments

where poplar is uncommon. N. Y. B. G. Nos. 3947, 3949, 4109, 6903, 7554.

*BADHAMIA CAPSULIFERA* (Bull.) Berk. Miss Charlotte B. Buckland has sent to me a specimen of this species collected at Mountain Lake, Virginia, in July 1933. The small fruiting is on moss growing on dead chestnut wood. The sporangia are sessile, more or less clustered but not heaped or superimposed, and measure about .5 mm. diam. The peridial walls are thin with little lime, but with veining or concentration of the lime in lines and ridges. The spores are characteristic without any ridges or bands; firmly clustered in groups of 8 to 20; strongly warted on the exposed surfaces but almost smooth on the inside. They are purple-brown in color and measure about  $12\mu$  when swollen in water to their globose shape. N. Y. B. G. No. 8166.

*BADHAMIA GRACILIS* Macbr. This seems to be a well marked center around which may be grouped certain forms that are probably common and widely spread in North America, and differing sufficiently from *Badhamia macrocarpa* (Ces.) Rost. to be regarded as distinct.

I have here now 11 collections by various collectors made in Colorado, Kansas, Florida, Maryland, New York, New Jersey? and Porto Rico, on habitats of *Yucca*, cactus, wood and bark. Among them is one labelled "*Badhamia panicea* (Fr.) Rost., on dead *Yucca*, Leyden, Colo., Feb. 5/10, E. Bethel, Denver, Colo." This is probably part of the collection described by Sturgis (Colo. Coll. Publ. Sc. Ser. 12: 438. 1913) as *Badhamia macrocarpa* (Ces.) Rost. The spores on this specimen do not have the bands and ridges, in addition to spines, that Sturgis refers to; nor do any of the specimens here have the coarse reticulum on the spores that Macbride & Martin mention in their description. The spores when dry assume a polyhedral shape with many faces and edges. Unless thoroughly wetted they will show shrinkage lines which may be mistaken for spore sculpture. When properly swollen in water, it will be seen that the spores are globose, not angular, with numerous small spines, but no bands, ridges, or reticulations.

All specimens here show, more or less, the small, subglobose, umbilicate sporangia, on rather firm but slender, yellowish stalks, which latter range from one-half to two-thirds the total height.

The peridium is thin with scanty lime. The capillitium is composed of delicate slender strands, much like that of *Badhamia foliicola* Lister. The spores are violet-brown, not very dark, spinulose with occasional blank areas, and measure 11–15  $\mu$ . The form is quite different from the rugged *B. macrocarpa* and the only similarity seems to be in the spores. N. Y. B. G. Nos. 1741, 1791, 5640, 5700, 6161, 6689, 6967, 8056, 8090, 8213, 8217.

*BADHAMIA OVISPORA* Racib. Three specimens on dog, horse, and rabbit dung collected in Germany and Saskatchewan, and developed in the laboratory at Toronto, Ontario, in 1935 and 1936, have come here from Dr. Roy F. Cain. The white, sessile sporangia are small, 0.03–0.04 mm. diam., sometimes confluent or extended into short plasmodiocarps. The sporangium wall is covered with dense deposits of white lime granules, though varying in amount to give a spotted or rugulose appearance. The calcareous capillitium has no hyaline threads and is often compacted at the base of the sporangium. The spores are violet-brown in color, ellipsoid, usually about  $9 \times 12 \mu$ , with many globose ones about 10  $\mu$  diam. A line of dehiscence is visible on many of the spores. N. Y. B. G. Nos. 8025, 8026, 8027.

*COMATRICHA IRREGULARIS* Rex. The extremities of the capillitium are usually pale. An interesting phase collected by Mr. Chabot at Brookville, Long Island, has the entire capillitium pale, so that the sporangia appear white when blown free from spores. Otherwise it is typical. N. Y. B. G. No. 1925.

*COMATRICHA RISPAUDII* Hagelstein. A typical collection was made near Hanover, New Hampshire, in August, by Mr. Eli Davis of London, Ontario, while in the company of Mr. Rispaud and myself, the occasion being the Foray of the Mycological Society of America. The species has been recorded heretofore only from Long Island and from near Ithaca, both in New York. N. Y. B. G. No. 4007.

*DIACHEA LEUCOPODIA* (Bull.) Rost. A large fruiting of var. *globosa* Lister was found near Plandome, Long Island, in July by Mr. Rispaud. The iridescent purple tints are not so evident in the globose sporangia, which are generally of a brownish-bronze color, but in the typical cylindrical sporangia, present with shorter, intermediate ones, the purple color is more pronounced. The vari-

ety is close to *Diachea bulbilosa* (Berk. & Br.) Lister, but may be distinguished readily when cylindrical sporangia are present. N. Y. B. G. No. 1910.

*DIACHEA RADIATA* G. Lister & Petch. A fructification apparently of this species has been received from Dr. Erdman West and collected at Gainesville, Florida, in June 1934. The crowded, globose sporangia, iridescent gray in color, are on grass and weeds, and seated on, or slightly imbedded in, a more or less continuous, white, calcareous hypothallus. There are no stalked sporangia. The sporangial walls are thin, membranous, colorless. In many of the sporangia the hypothallus is drawn in to form a short, white columella; in others there is a yellow, membranous one; in some it is lacking. The dark capillitium springs from the columella or from the central part of the sporangial base when the columella is indefinite. The spores are pale violet-gray, distinctly spinulose, and measure about  $11\ \mu$  diam.

This specimen agrees with the description and figures of *D. radiata*. The color of the plasmodium, reported as yellow, is unknown, but I do not regard the specimen as representing a sessile phase of *Diachea leucopodia* (Bull.) Rost. or *Diachea bulbilosa* (Berk. & Br.) Lister, as sessile sporangia of those species are usually scattered and accompanied by stalked ones. The crowded habit and absence of stalks indicate *D. radiata*. N. Y. B. G. No. 5180.

*DIACHEA SPLENDENS* Peck. Five gatherings were made in Pike County, Pennsylvania, in August, and I have also before me collections from New Jersey, Virginia, Mississippi, and part of the type collection of Peck from New York. Widely separated, they are remarkable for their constancy in all characters.

In earlier editions of the British Monograph, the form was regarded as a species, but in the 3rd edition Miss Lister reduced it to a variety of *Diachea bulbilosa* (Berk. & Br.) Lister on gatherings of the latter from Ithaca, New York, made by W. C. Muenscher (not Muenschen). F. B. Wann and W. C. Muenscher recorded the collection of *D. splendens*, but not *D. bulbilosa*, in their paper "A Preliminary List of the Myxomycetes of the Cayuga Lake Basin" (Mycologia 14: 38-41. 1922) which included the Ithaca region. In 1922 they distributed to various institutions



including the British Museum and the New York State Museum at Albany, a series of 50 specimens from collections made within the area covered by their papers. This is Fascicle 1 of North American Myxomycetes as titled by them. I have examined the collection in the New York State Museum, and specimen No. 8, from Enfield Gorge near Ithaca, is labelled *D. splendens*. This is not *D. splendens* but is *D. bulbillosa* and the same as others described in a later paragraph. There are no other specimens of either species. Dr. Muenscher writes to me that he is certain that all the sets distributed were labelled alike, and that all the material in No. 8 was the same; also, that an earlier specimen was sent to Miss Lister, but that it came from a gathering different from No. 8 of the series. He has courteously sent to me a specimen from Hamilton County, New York, named *D. splendens* and numbered 53 of Fascicle 2 of the same Exsiccatae. This is also *D. bulbillosa* and not *D. splendens*.

The two species mentioned, together with var. *globosa* of *Diachea leucopodia* (Bull.) Rost., are separated mainly by spore characters, but there are other differences between them. The spores in all specimens before me are uniformly from 7.5–8.5  $\mu$  diam., and the color is some shade of violet-gray. The globose variety of *D. leucopodia* is usually associated, more or less, with cylindrical sporangia of the typical form. The spores are faintly marked with numerous minute spines evenly distributed. *D. splendens* has large, globose sporangia, in diameter almost half again as much as those of the other species. The color is always a beautiful blue, not exactly iridescent, but scintillating under the lens, rarely if ever seen in the others. The stalk is stout, white, cylindrical, filled with rounded lime granules. The spores—every spore in every sporangium—have the long, cylindrical processes or protuberances, in height up to 1.5  $\mu$ , and truncate or flared at the tops. These are not spines nor warts, and nothing like them appears on the spores of the other two species. There can hardly be any intermediate stages between two such diametrically opposed forms of spore sculpture, and while warts and ridges of various sizes also appear commonly on spores of *D. splendens*, they do not lessen the importance of the cylindrical protuberances as a specific character.

I have here only one specimen, collected in Florida by Dr. Erdman West, that I can regard as typical *D. bulbillosa*. The globose sporangia are much smaller than those of *D. splendens* and are iridescent gray in color. The stalk is rather slender, tapering at the top, white at the base, and reddish-brown for the greater part upward. The spores are sparsely sown with dark, scattered spines, often only four or five across the hemisphere. When observed on edge, the spines are seen to be pointed and about  $0.05\ \mu$  in height. They are more prominent than those on the specimens later described, and the spore color is more grayish. The stalk and clavate columella is filled with large rhombs of crystalline lime. It may well be that this crystalline lime is an important feature of the species.

I have here also two specimens personally collected in Schoharie County, New York, another from Massachusetts, and two from Luzon, Philippine Islands, which with the two previously mentioned as collected by Dr. Muenscher at Ithaca and in Hamilton County, New York, constitute a series all in the same category and alike except in minor, unimportant details. The globose sporangia are smaller than those of *D. splendens*, and there are no cylindrical sporangia or any that approach *D. leucopodia* in shape. The color is not the beautiful blue of *D. splendens*, but ranges from a dingy blue to brown or gray and often iridescent. The stalks taper at the tops and end in clavate columellae, filled throughout with rounded lime granules. The capillitium is denser in some specimens than in others. The spores have dark, scattered spines which vary in the number on the hemisphere, but are not more than eight in a line across. They are grayish-violet in color and measure  $7.5\text{--}8.5\ \mu$  diam. These seven collections are regarded as *Diachea bulbillosa* on the spore markings, the clavate columella, and the absence of cylindrical sporangia.

In all of these specimens regarded as *D. bulbillosa* there is not a single sporangium among the many examined that has spores with markings similar to those on the spores of *D. splendens*. Also, the difference in appearance between the two is so pronounced that they can be separated with the unaided eye. From the large series before me, I am convinced, and agree with the opinion of other American students, that *Diachea splendens* Peck

should be regarded as a distinct species. Many specimens in the Herbarium of the New York Botanical Garden.

*DIDERMA SIMPLEX* (Schroet.) Lister. As noted in the introduction, the species appeared in great abundance during the season so that extensive collections were made on Long Island and in Pennsylvania. Likewise in New Hampshire, but during our stay there with constant rains, the fruitings were generally imperfect and unsuitable for study. The form develops on ground material in wet places, and this tends to produce many poor fruitings. With the large amount of new, good material available, it has been possible to make a broader study of the species as it occurs here. In earlier collections that I have made there is nothing to alter the conclusions expressed.

The species is well described in the British Monograph and little amendment is required for the present material. It is very variable in shape, color, and habit of the sessile sporangia and plasmodiocarps, and also in spore characters. The spores range from  $7.5\text{--}12\ \mu$  diam., and the spines or warts vary in their visibility. There is no hypothallus except in a few instances where its presence is due more to imperfect fruition. The so-called hollow columellae are not columellae at all as the species does not form columellae in any specimens that I have seen from North America. They are present in all phases of the species, and depend entirely upon the shape of the sporangia and plasmodiocarps. In flattened sporangia they are hardly evident and the floors are firmly attached to the basal habitat. In rounded or subglobose sporangia, the floors are attached only at the peripheries and the centers are raised to approach the convex tops as uniformly as possible. In many instances the sporangium may be separated from the habitat and the concavo-convex shape observed. The formation is seen in the highest degree in the longer, vertically extended, or contorted sporangia and plasmodiocarps that form heaped or clustered groups. The plasmodium tends to form more or less flattened or subglobose sporangia with uniformly separated upper and lower walls. These walls conform to the prevailing condition, which will often be a small quantity of liquid exuded by the plasmodium below the basal part of the wall and around which the sporangium will form. The formation may be further modified

by pressure from adjoining sporangia when crowded or heaped. The formations therefore are not columellae formed within the sporangia, but modifications of the sporangial floors, and similar to those in sessile sporangia of other species that form around small plant stems or on the edges of leaves; only in such cases the cavities are filled by the habitat.

Lister says that the columella of *D. simplex* is indefinite and rugose, or convex. I have no examples of European developments, but if those do show a solid columella, it might be that our American forms are specifically distinct.

Var. *echinulatum* Meylan appeared abundantly in Pike County, Pennsylvania, in September, associated with other phases. The sporangia are well rounded, show often the raised floors, and are bright yellow in color so that they can be easily recognized in the field. The spores are strongly spinulose, more so than usual in the typical form, but prominently marked spores appear also in collections of other phases. Many specimens in the Herbarium of the New York Botanical Garden.

*DIDERMA TREVELYANI* (Grev.) Fries. A small, perfect fruiting was found by Mr. Lloyd G. Carr in Augusta County, Virginia, in September, and is perhaps the first collection from States along the Atlantic Coast. The reddish-brown sporangia, dehiscing in the petal-like manner, are seated on short reddish stalks. The outer sporangium wall is beset with scattered plates of lime, and between the outer and inner walls is a closely compacted layer of coarse, partly crystalline masses of lime. Many of the sporangia have a minute, subglobose, calcareous columella which is often eccentric; others are free from it. The capillitium has numerous dark, bead-like thickenings. The spores are spinulose and measure about  $12\mu$  diam. They are not dark violet-brown as usual, but paler and more grayish, and have a pale area of dehiscence. N. Y. B. G. No. 8237.

*DIDYMIUM CRUSTACEUM* Fries. Two perfectly developed and typical fruitings of this rare and curious species were found in Wayne County, Pennsylvania, in September. The sporangia are irregular in shape, sometimes confluent, and closely aggregated. Many of them have a yellow, membranous pseudo-stalk, an elongation of the membranous hypothallus. The membranous wall or

peridium enclosing the capillitium and spores, as mentioned in descriptions, is practically non-existent, or at least not as a continuous membrane. The capillitium and spores are covered with a dense layer of large, loose, stellate crystals of lime and crystalline masses of lime. Surrounding all this, and separated therefrom, is another thin, delicate crust of large, loose, stellate crystals of lime without any admixture of crystalline masses. This crust is smooth on the outside, but irregular with projecting crystals on the inside.

The outer crust of pure lime crystals is here regarded as distinct and forming separately from the sporangium proper. It is very fragile, crumbling and disappearing with the slightest disturbance. When perfectly developed it is not attached to the sporangium but covers it like an inverted jar. It seems to form by the rapid evaporation and crystallization of the saturated medium containing lime in solution which is discharged by the plasmodium when it divides to form sporangia. The outer side would be smooth from surface tension. As sporangial formation proceeds within, and with consequent contraction, there would be a layer of the liquid between the outer formed crust and the forming sporangium. By evaporation, further crystalline deposits of lime would be made wherever the liquid touched, principally on the forming capillitium and spores; on the stalk, or from it to the outer crust along the habitat base; or as connecting masses between the sporangium and outer crust. These deposits may be observed in many sporangia. The confined liquid also plays an important part in the shaping of the irregular sporangia, conforming them to its pressure or the shape of the outer crust.

I have also received another specimen of the species collected by Dr. C. L. Shear in the Shenandoah National Park, Virginia, in September 1934. N. Y. B. G. Nos. 4003, 4229, 8063.

*DIDYMIUM MINUS* Morg. This is no more than a small phase of *Didymium melanospermum* (Pers.) Macbr. It forms plasmodiocarps at times and such a collection was made at Plandome, Long Island, in July, on a beech leaf associated with normal, stipitate sporangia on oak leaves. The sporangia are small with small spores about  $8\mu$  diam. The sessile plasmodiocarps are rounded or elongated, and flattened or depressed in the centers,

with similar spores. There is no columella in the plasmodiocarps. They are much like those of *Didymium anellus* Morg. and may be mistaken therefore. *D. anellus* is usually smaller, thinner and flatter, and has a tendency towards circumscissile dehiscence, often well marked. The peridium of the latter is thinly sprinkled with lime crystals so that it appears in places as an iridescent membrane. The dehiscence in *D. minus* is more irregular, and the spores often show various sizes in one sporangium, an indication of abnormality. N. Y. B. G. Nos. 1916, 1944.

*DIDYMIUM STURGISII* Hagelstein. Another collection was made in Wayne County, Pennsylvania, in June. In an earlier note about this species (*Mycologia* 29: 397. 1937), I gave the thickness of the plasmodiocarps as 1-2 mm. This was a typographical error. It should have been 0.1-0.2 mm. N. Y. B. G. No. 4216.

*FULIGO MUSCORUM* Alb. & Schw. We have searched high and low for this species for a number of years, and were not rewarded until one September day when we entered a small, wet, spagnum bog in Pike County, Pennsylvania, and found the first and largest aethalium on spagnum moss. Following our usual practice when a desirable form is located, we searched the surrounding area in widening circles until others were found, so that after several visits we had about 50 aethalia in all. Also, many developing plasmodia enabled me to observe and study them from emergence to full maturity. The yellow plasmodium, on emergence from the substratum of the ground, commenced to divide into several branches, usually four or five, appearing like an outstretched hand and about that size. Each of these branches became the base for one or more aethalia after separation from the other branches. The next day after emergence the branches had separated and the plasmodium was drawn up in rounded masses a foot or two apart. On the following day each mass was developing an immature aethalium, much contracted in size, and surrounded by a watery liquid discharged by the plasmodium. On the third day maturity was complete, with the aethalia still further reduced in size, or divided into several smaller aethalia separated by a few inches. Earlier division into smaller aethalia was not observed. From

6 to 10 aethalia may therefore be located in the limited area where the plasmodium emerged.

The pulvinate aethalia range in size from a few millimeters to about 3 cm., usually about 6–10 mm. When normally developed under proper drying conditions, so that all the lime in solution in the watery envelop is deposited on the aethalium, the color will be orange-yellow. If disturbed, or the liquid joins the water of the wet substratum, there will be less lime deposited and the color will be greenish or gray. The lime-knots in the capillitium are yellow or sometimes white, numerous, and irregular or branching. The spores are violet-brown, spinulose, and measure 10–11  $\mu$ . The form cannot be confused with small, solitary developments of *Fuligo septica* (L.) Weber, and is recognized by the many aethalia, the habit, and the moist habitat.

There are here, also, several collections made by the late Prof. R. Thaxter in Maine and New Hampshire many years ago. These and the Pennsylvania specimens differ from a specimen from North Wales only in that the outside appearance is not as smooth. Our specimens follow more closely the convolutions of the interwoven sporangia of which the aethalia are composed. N. Y. B. G. Nos. 4106, 7695, 7937, 7938, 7939.

*FULIGO SEPTICA* (L.) Weber. Two aethalia, evidently from the same plasmodium, and collected in the Bottomless Pit, near Hanover, New Hampshire, in August, are noteworthy as having outer and inner lime of an apple-green color. N. Y. B. G. Nos. 3985, 3986.

*HEMITRICHIA ABIETINA* (Wigand) Lister. A large collection of the species was made in Pike County, Pennsylvania, in early September. Many of the sporangia show well developed stalks, some of which are almost half the sporangial height. During the past year further collections of the species have come to my table from Dr. C. L. Shear, made at Ball's Bluff, Virginia; from Dr. J. A. Stevenson from the Shenandoah National Park, Virginia; and from Dr. I. F. Lewis made in Albemarle County, Virginia. The form is not rare, apparently, in the mountains of the eastern United States. N. Y. B. G. Nos. 4283, 8060, 8066, 8174, 8177.

*HEMITRICHIA INTORTA* Lister. I had almost given up hope that another authentic specimen of this species would ever come



to light again from the eastern States, when it appeared among some undetermined material sent to me by Dr. D. H. Linder of the Farlow Herbarium. It was collected by the late Prof. R. Thaxter, at Waltham, Massachusetts, in November 1885, six years before the species was proposed. With the lapse of time, one of the most important characters of the species has lost emphasis in descriptions. The capillitium is not like that of the usual *Hemitrichia*, that is, a branched net. It consists of several very long loops, attached at both ends to the base of the sporangium, much twisted and with twisted projections that appear as free ends but are not. If these loops could be untwisted and stretched out they would reach to a length of 20 mm. or more. The specimen shows practically no branching or netting between the loops. The capillitium has more than three close, prominent spirals, and is minutely spinulose. The stalk is solid without spore-like cells; the wall is single; and the spores measure 8.5–9.5  $\mu$  diam. N. Y. B. G. No. 8081.

*LINDBLADIA EFFUSA* (Ehr.) Rost. The variety *simplex* Rex is not uncommon in northeastern Pennsylvania. In one collection many of the sporangia show traces of thickenings in the upper part, indicating the close approach to *Cribraria argillacea* Pers. Both variety and typical form are often associated with *C. argillacea* on the same wood. N. Y. B. G. Nos. 4288, 4292, 4297.

*MARGARITA METALLICA* (Berk. & Br.) Lister. A small fruiting of the species was collected on Balch Hill, near Hanover, New Hampshire, in August, and another sent here for verification was collected by Mr. Lloyd G. Carr in Rockingham County, Virginia, in October. N. Y. B. G. No. 4008.

*ORCADELLA OPERCULATA* Wing. The small size and scattered habit prevent detection of the species in the field. A few typical sporangia were found on oak bark associated with another species, and collected on Moose Mountain, near Hanover, New Hampshire, in August. N. Y. B. G. No. 4058.

*PHYSARUM AENEUM* R. E. Fries. I have received from Mr. Travis E. Brooks a specimen on grass and stems collected by him in Geary County, Kansas, in June 1937. It consists entirely of elongated straight or slightly curved plasmodiocarps on narrow bases, yellowish-brown in color, about .3 mm. high and up to



3-4 mm. in length. There are double walls; the outer cartilaginous, brittle, shining, with included deposits of lime granules; the inner thin, membranous, entirely separated from the outer wall and exposed when the latter breaks away at the top. The inner wall is sparsely coated with rounded deposits of brown lime. The capillitium has numerous small, rounded, dark brown lime-knots, and the spores are pale, brownish-violet, measuring  $7-8\ \mu$  diam. N. Y. B. G. No. 8198.

*PHYSARUM DIGITATUM* Farquh. & G. Lister. Collected by Dr. John A. Stevenson in Anne Arundel County, Maryland, in June 1937. The gathering is typical. The species has been rarely reported from territory along the Atlantic Coast of North America, if at all. N. Y. B. G. No. 8285.

*PHYSARUM GLOBULIFERUM* (Bull.) Pers. Common in eastern North America and developing on all sorts of dead wood which explains, somewhat, the occurrence of so many variations. The globose, persistent capillitium; the columella; the calcareous stalk; and the rounded lime-knots are usually together as constant characters. The capillitial lime, when examined by transmitted light, is often a shade of pale yellow. An abnormal, but perfect development, on birch bark was collected in Pike County, Pennsylvania, in July, and has an almost *Badhamia*-like capillitium with numerous, large, white, angular or branching lime-knots, often densely aggregated. The spores are pale and small,  $7-8\ \mu$  diam. N. Y. B. G. No. 4391.

*PHYSARUM LATERITIUM* (Berk. & Rav.) Morg. The scarlet phase with yellow lime-knots and red centers is quite common as we find it frequently, and usually on dead leaves of *Corylus* (hazel). The smaller, yellowish-brown phase mentioned in my Long Island paper (*Mycologia* 28: 603. 1936) was found during the season in New Hampshire and Pennsylvania and again on Long Island. It has also been collected in other sections in earlier years. It is probably the form described as *Physarum Brauni-anum* De Bary, and figured by Lister on plate 61 as fig. *d*. Many specimens in the Herbarium of the New York Botanical Garden.

*PHYSARUM LISTERI* Macbr. The rules of nomenclature preclude the use of the name *Physarum luteo-album* Lister. A representative collection on leaves was made by Mr. Lloyd G. Carr

in Augusta County, Virginia, in September. The sporangia are subglobose, somewhat umbilicate beneath, stalked, and deep orange in color. The stalk is stout, smooth, concolorous with the sporangium or paler, and densely charged with lime granules. The sporangia have double walls; the outer orange-yellow, dense with deposits of yellow lime granules; the inner membranous, pale yellow or white, with scantier lime. The walls separate and the outer breaks away at maturity. The capillitium has pale yellow threads with pale yellow, spindle shaped or branching lime-knots, and is attached to a large, yellow, subglobose columella which latter is surrounded by a collar with persistent tufts of the capillitium after the walls have broken away. The spores are purplish-brown, strongly spinulose with long spines, and measure  $12\ \mu$  diam.

It is strange that in descriptions of the species no particular mention is made of the prominent double wall, but this may have been poorly defined or absent in earlier collections which have been rarely reported. Our only other specimen was found by Dr. W. C. Sturgis in Colorado, in August 1911 (Colo. Coll. Publ. Sc. Ser. 12: 439. 1913), and has a single, membranous, lime-less wall, with little lime in the capillitium. N. Y. B. G. Nos. 7388, 8238.

**PHYSARUM PENETRALE** Rex. The form is not rare but not often collected because of the small fruitings and the habit of disintegrating rapidly. We found it during the past season on Moose Mountain, near Hanover, New Hampshire, in August, and twice in Pike County, Pennsylvania, in June and July. Mr. Robert O'Connor found it also during the past season, on Staten Island, New York. N. Y. B. G. Nos. 4006, 4347, 4378.

**PHYSARUM SERPULA** Morg. The species has come to me determined as *Badhamia decipiens* (Curt.) Berk. which is excusable, as formerly it was often regarded a phase of the latter, and the descriptions and characters of the two differ so little that it is possible to misunderstand them. The principal differences to be noted in the field are habit and habitat. *B. decipiens* forms scattered sporangia and plasmodiocarps in few and small developments on wood. *P. Serpula* is on ground leaves, twigs, etc., in numerous, small, densely aggregated colonies. In our eastern material the capillitial lime of *P. Serpula* is often white, and the wall is rougher

than in *B. decipiens*. *P. Serpula* also resembles the form heretofore known as *Physarum variabile* Rex var. *sessile* Lister, but in the latter the spores are different. *P. Serpula* is not common but when located is usually abundant. At Middleburg, in Schoharie County, New York, in August 1935, thousands of colonies appeared in a small area, all reaching maturity at the same time. N. Y. B. G. Nos. 3321, 8234.

**PHYSARUM VARIABLE** Rex. Several small collections have been made in recent years in New Hampshire and Pennsylvania, and also at Mountain Lake, Virginia, the last by Miss Charlotte B. Buckland. There is practically nothing of importance to distinguish the form from *Physarum sulphureum* Alb. & Schw., so, following Miss Lister, I am regarding it now as a phase of the latter species. The specimens described as *P. variabile* var. *sessile* (Mycologia 28: 608. 1936) are tentatively regarded as *Physarum sessile* Brandza. N. Y. B. G. Nos. 3366, 4245, 4272, 8150.

**PHYSARUM VERNUM** Somm. One of my associates, Mr. Leon J. Chabot, while on a visit to Whitefield, New Hampshire, in July, found a rather large and perfect development on wood consisting of many long, branching and netted plasmodiocarps, with occasional sessile sporoangia, which is exactly like similar fruitings of *Physarum cinereum* (Batsch) Pers., only more robust and with different spores. Many of the plasmodiocarps are from 5–10 mm. in length, and stout in proportion. The spores are dark, violet-brown—much darker than in specimens from the Swiss Alps—strongly marked, and measure 7.5–8.5  $\mu$  diam. *P. cinereum* has spores that are pale and almost smooth. The spores are too small to reconcile the specimen with *Physarum compressum* Alb. & Schw., and similar plasmodiocarps are not formed in that species. Another small development was found by Mr. Rispaud near Hanover, New Hampshire, in August. I am regarding both collections as *P. vernum* on the resemblance to *P. cinereum* and the dark spores. N. Y. B. G. Nos. 4021, 4084.

**PHYSARUM VIRESCENS** Ditmar. Another species that appeared in abundance in 1937 on Long Island and in New Hampshire and Pennsylvania. Var. *nitens* Lister was also found in New Hampshire and Pennsylvania, and, as usual, in small colonies. We have collected the variety in earlier years on Long Island and in

other parts of New York, so that there are here about a dozen specimens. The variety is very different from typical *P. virescens* which latter has heaped, often irregularly ovoid sporangia that may be confluent or seated on a membranous hypothallus. The sporangia of var. *nitens* are larger, subglobose, gregarious and sometimes crowded but never heaped. The color and general appearance is also different. The form is constant and so easily distinguished from typical *P. virescens* that, in my opinion, it should be regarded as a distinct species.

Dr. Sturgis examined the type specimen of *Physarum luteolum* Peck in the New York State Museum at Albany (Trans. Conn. Acad. Arts & Sci. 10: 470. 1900) and regarded it as probably the same as *P. virescens* var. *nitens*. I have also examined the specimen, and while little remains, I agree with Dr. Sturgis. The heretofore recognized variety is now regarded here as *Physarum luteolum* Peck. Many specimens in the Herbarium of the New York Botanical Garden.

*STEMONITIS NIGRESCENS* REX. This is not common hereabouts, and I have never found anything on wood that I could reconcile therewith. Three collections on leaves and moss were made in sphagnum bogs in Pike County, Pennsylvania, in September, and all agree well with an authentic specimen from Dr. Rex in the Herbarium of the New York Botanical Garden. The form is within the group surrounding the variable *Stemonitis fusca* Roth, if judged by anatomical characters alone, and may be a variety thereof as Lister says. However, the plasmodium of *S. fusca* invariably inhabits decaying wood, and if further field observations indicate that the plasmodium of *S. nigrescens* thrives in the substratum of the soil, I will be more firmly convinced that it deserves specific rank, considering then also differences in characters of size, color, spores, and surface net. N. Y. B. G. Nos. 4105, 4364, 4397.

*TRICHIA ERECTA* REX. A specimen has been received from Miss Charlotte B. Buckland collected by her at Mountain Lake, Virginia, in August 1933, which has sporangia with mottled peridia, and seated on stout, dull-brown stalks. Another specimen received from Mr. William W. Ray collected in Monroe County, Pennsylvania, has similar sporangia on black stalks. In both

specimens the elaters of the capillitium are studded with numerous minute spines and end in short, tapering tips. The spores in each measure  $11-13\ \mu$  diam. The form cannot be distinguished from *Trichia botrytis* (Gmel.) Pers. by the mottled peridium alone, as *T. botrytis* when found in the simple, sporangiate phase here in the East has usually the same mottled peridium. The elaters of the capillitium and the spores of *T. erecta* and *Trichia subfusca* Rex are often similar except for the spines on the elaters of *T. erecta*. *T. subfusca* does not have the mottled peridium. *T. botrytis* has elaters with long, smooth slender tips without spines, and the spores are smaller than in the other two species. N. Y. B. G. Nos. 7521, 8156.

*TRICHIA LUTESCENS* Lister. While at Mountain Lake, Virginia, this past summer, I was provided with a small portion of a gathering of this species made by Mr. Lloyd G. Carr in Augusta County, Virginia, in July. The membranous peridial walls of the sessile sporangia are olivaceous-yellow in color, translucent, and without any granular deposits whatever. The capillitium is somewhat branched and netted, but such conditions are not unusual among the Trichias, and particularly in *T. lutescens* as Lister remarks. There are four spirals on the elaters, and the spores are warted. N. Y. B. G. No. 8196.

*TRICHIA SUBFUSCA* Rex. Two collections of this species were made; one on Moose Mountain, near Hanover, New Hampshire; and the other in Pike County, Pennsylvania, both in August. The form resembles somewhat the simple sporangiate phase of *Trichia botrytis* (Gmel.) Pers., but does not have the mottled peridium as the dark, granular deposits are evenly distributed. The stalks are short and stout. The elaters of the capillitium have prominent spirals without spines, and the spirals wind almost to the abruptly ending tips. N. Y. B. G. Nos. 4059, 4374.

THE NEW YORK BOTANICAL GARDEN

## NOTES AND BRIEF ARTICLES

### CORDYCEPS MILITARIS AND ISARIA FARINOSA

Dr. T. Petch has published an interesting paper under the above title in the Transactions of the British Mycological Society 20: 216-224. 1936. As a result of his studies he has found that *Isaria farinosa* has no relation to *Cordyceps militaris*, although such a relationship was claimed by Tulasne (Selecta Fungorum Carpologia 3: 5. 1865). This statement will come as something of a shock to those of us who have been collecting *Isaria farinosa* and listing it as the conidial stage of *Cordyceps militaris*. A full discussion of this matter is contained in Dr. Petch's excellent paper.—F. J. SEAVER.

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The Journal of Agriculture of the University of Puerto Rico, volume 21, no. 3, consists of "A bibliography of mycology and phytopathology of Central and South America, Mexico and the West Indies," by Jose I. Otero, Librarian, and Mel T. Cook, Plant Pathologist. The bulletin consists of 241 pages, and includes all the available titles on the subjects indicated above. The authors are especially desirous of being notified of any omissions or corrections. This bibliography will be invaluable to those who are working on the fungi of the tropics.—F. J. SEAVER.

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### THE FUNGI OF CYPRUS

The British dependency of Cyprus is an isolated island in the eastern Mediterranean 140 miles in extreme length and 60 miles in greatest breadth whose surface is diversified by mountains and plains. Its native tree flora, now greatly reduced, consists of a few conifers, one species of oak, the olive and the carob whose products have been the chief commercial exports of the island. The last-named tree, related to our Judas tree and *Cassia*, produces edible pods called alcaroba beans, locust pods, and St. John's bread because there is a reasonable belief that John the Baptist

lived on locust pods and wild honey instead of sweetened grasshoppers.

The reported average maximum temperature of July is nearly 98° F. and the average minima of February nearly 42° F.; the rainfall varies greatly but averages about 20 inches.

The first and only list of this interesting island's fungi has been made by Mr. R. M. Nattrass, the official plant pathologist, covering his observations and collections during the last six or seven years. It is a locally and very creditably printed booklet of XVI + 87 pages, 15 plates and a map. Ninety-one species of rusts, including one new species, lead in point of numbers; the hyphos come next with 87 species; sixth are the smuts with 19 species and excepting potato-scab the bacteria are fewest with 5 species. In all the groups except the agarics and their relatives pathogenic species predominate altho as elsewhere the saprophytes are probably more numerous.

In respect to hosts, the orange and lemon harbored 20 species; plum and cherry 17; potato 14; tomato and durum wheat 11 each and carob 7. New species described by Mr. Nattrass are:

*Phaeodothis Hyparrheniae*, on *Hyparrhenia hirta*

*Sporocybe cypria* imp. of *Petriella asymmetrica* Curzi v. *cypria*  
on *Populus nigra*

*Phyllachora Ravennae*, on *Erianthus ravenna*

*Uromyces Aeluropodis-repentis*, on *Aeluropos repens*

*Alternaria Cichorii*, on *Cichorium intybus*

*Hendersonula cypria*, on *Prunus armeniaca*

*Petriella asymmetrica* Curzi var. *Cypri* Nattr., on *Populus nigra*

*Uromyces vesicatorius* (Bubak) Nattr., on *Leontice leontopetalum*

*Microdiplodia Warburgiana* (Reichert) Nattr., on *Citrus limonum*.

Types or co-types as well as examples of the other fungi are deposited in the herbarium in the island Department of Agriculture and also with the Imperial Mycological Institute at Kew, England. Exemplifying the cosmopolitanism of fungi species-names familiar to American mycologists recur on every page; at a guess considerably more than half the species are represented in American herbaria.—JOHN DEARNESS.

THE FUNGI<sup>1</sup>

The above is the title of a new textbook recently issued by H. C. I. Gwynne-Vaughan, Professor of Botany in the University of London, and B. Barnes, Head of the Department of Biology, Chelsea Polytechnic, London. The book is a second edition of a volume on fungi published by the senior author in 1922, but covering a much wider scope. The introductory chapter deals with the general characteristics of the fungi. The second takes up in detail the physiology of the fungi including saprophytism, parasitism, and symbiosis, and a discussion of their reaction to stimuli of various kinds.

After a brief discussion of the Myxomycetes the body of the work is devoted to a consideration of the morphology of all of the fungi from the Phycomycetes to the higher Basidiomycetes. The closing chapters deal with the cultivation, staining, and microscopic examination of the fungi. The book consists of 449 pages of text and more than 300 figures.

While the objective of the book seems to be excellent, it is nevertheless subject to some rather serious criticisms. Naturally the writer, having devoted many years to researches on the Discomycetes, was interested in the authors' treatment of this group. It is not all that could be expected. On page 200 the authors, after describing the methods of dehiscence of the ascus, state "*The distinction between these modes of dehiscence has been used as a basis of classification and is of importance in indicating affinities.*" After making this statement the authors entirely ignore this character and adopt a classification which is essentially that used by Schröter and Lindan in Engler and Prantl's *Pflanzenfamilien* in 1897, bringing together in the same group the inoperculate Geoglossaceae and the operculate Helvellaceae as was done by them at that time.

Naturally in writing a text one is free to adopt any sort of classification which suits his or her fancy, provided this classification is not based on a misstatement of facts. To determine this let us examine some of their statements more in detail. On page 207 in dealing with the Pezizaceae the authors state "*The ripe asci*

<sup>1</sup> Published by Macmillan Co., New York.



do not project above the level of the disc as they do in the *Ascobolaceae*." This is an old belief which has long since been exploded by critical students of the Discomycetes.

Again on page 216 in dealing with the *Ascobolaceae* the authors state "The family is distinguished from the *Pezizaceae* by the usually multiseriate arrangement of the spores and by the fact that the ripe asci stand well above the level of the hymenium before their spores are discharged." The biseriate arrangement of the spores is common to both the *Pezizaceae* and those which these authors include in the *Ascobolaceae*. In fact, it varies in a given species, the spores often being uniseriate when young and biseriate when mature. This character is of no diagnostic value whatsoever.

The protrusion of the asci in the *Pezizaceae* has already been referred to. It was once believed that the *Ascobolaceae* were characterized by their protruding asci. This was due to the fact that in some species of *Ascobolus* the asci were very large and the spores dark colored so that the protrusion of the ascus was more conspicuous. It is now known that this character is equally common to both the *Ascobolaceae*, as treated in this work, and the *Pezizaceae*, as has been pointed out repeatedly in mycological literature.

Again on page 217 the authors state "The spores are brown or violet in *Ascobolus*, *Saccobolus* and *Boudiera*." This is again apparently a perpetuation of an error by Schröder and Lindau in Engler and Prantl's *Pflanzenfamilien* in bringing together in the same genus *Boudiera* the colored spored *Ascodesmis microscopica* and the hyaline spored *Boudiera areolata*, which have no close relationship. This again has been pointed out in mycological literature more than twenty years ago. In reading over their treatment of the Discomycetes one might get the impression that the classification used was adopted for class use thirty-five years ago and published without revision, and apparently without any attempt to review the literature of the subject which has been issued during the intervening period.

However, it must be borne in mind that the senior author of this text is a cytologist and not a taxonomist, and we trust that her cytological details are more up to date than her systematic

treatment. With all the work on hybridizing fungi that has been done in the past ten years it is to be regretted that the authors did not see their way clear to discuss this important subject. Overlooking some of these glaring misstatements and omissions we may yet say that the general makeup of the book is excellent and it will doubtless find its way into many classes as a textbook of general mycology.—F. J. SEAVER.

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